

University of Alberta Library



0 1620 2852432 8

University of Alberta

Examining Committee

Metabolic regulation of global histone acetylation in the
yeast *Saccharomyces cerevisiae*

Dr. Luis Schling, Biochemistry

by

Dr. James R. Smith

R. Magnūs Nesbitt Friis

Dr. D. Alan Anderson

Dr. James R. Smith, University of Alberta

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Biochemistry

©Robert Magnus Nesbitt Friis

Spring 2010

Edmonton, Alberta

Permission is hereby granted to the University of Alberta Libraries to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only. Where the thesis is converted to, or otherwise made available in digital form, the University of Alberta will advise potential users of the thesis of these terms.

The author reserves all other publication and other rights in association with the copyright in the thesis and, except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatsoever without the author's prior written permission.

University of Alberta

Metabolic regulation of global histone acetylation in the
yeast *Saccharomyces cerevisiae*

by

R. Magnus Westhoff-Fries

Thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Botany

Robert Magnus Westhoff-Fries
Spring 2010
Edmonton, Alberta

The following thesis was submitted to the University of Alberta in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It is hereby certified that the thesis is the work of the author and that it has not been submitted for publication elsewhere in whole or in part. The author has also submitted a copy of the thesis to the University of Alberta Library for deposit and preservation.

The author grants to the University of Alberta a non-exclusive license to use the thesis in whole or in part for the purpose of research and scholarship. The author also grants to the University of Alberta the right to reproduce the thesis in whole or in part for the purpose of research and scholarship.

ABSTRACT

Examining Committee

Dr. Michael C. Schultz, Biochemistry

Dr. Luis Schang, Biochemistry

Dr. James R. Smiley, Medical Microbiology and Immunology

Dr. D. Alan Underhill, Oncology

Dr. James R. Davie, Biochemistry and Medical Genetics, University of Manitoba



Digitized by the Internet Archive
in 2026 with funding from
University of Alberta Library

<https://archive.org/details/0162028524328>

ABSTRACT

Nutrient metabolism exerts direct effects on transcriptional regulation and cell signaling. Herein we highlight the interplay between glucose metabolism and modules involved in transcriptional regulation.

Little is known about what enzyme complexes or mechanisms control global acetylation of nucleosomal histones. Here we show that glucose induces overall acetylation of H3 K9, 14, 18, 27 and H4 K5, 8, 12 in quiescent yeast cells mainly by stimulating two KATs, Gcn5 and Esa1. The picNuA4 (Esa1) and SAGA (Gcn5) complexes control glucose-induction of overall acetylation through an untargeted mechanism. Genetic and pharmacological perturbation of carbon metabolism, combined with ^1H -NMR metabolic profiling, revealed that glucose induction of KAT activity directly depends on increased glucose catabolism. Cellular energy balance is central to this regulation: the magnitude and duration of the acetylation response increase with the amount of glucose given to cells, and depletion of ATP reduces histone acetylation. Furthermore, after removal or depletion of extracellular glucose, histone acetylation levels decline more rapidly in p^0 cells, which lack mitochondrial DNA (mtDNA) and therefore respiratory capacity, than in p^+ cells with mtDNA. We conclude that direct metabolic regulation of globally acting KATs can be a potent driving force for reconfiguration of overall histone acetylation in response to a physiological cue.

Transcriptional profiling studies of p^0 cells revealed additional links between the metabolic state of cells and regulation of transcription. During growth on glucose, expression

of genes required for the metabolism of alternative carbon sources is repressed. In the absence of glucose this repression is lifted in ρ^+ but not ρ^0 cells. Repression is not lifted in ρ^0 cells because glucose signaling is misregulated. The transcriptional defects displayed by ρ^0 cells could be attributed to an inability to down regulate protein kinase A (PKA) activity in ρ^0 cells.

Consistent with this, hyperphosphorylation of the transcription factors Ume6 and Msn2 upon glucose removal, which can be blocked by PKA signalling, is blocked in ρ^0 cells. Additionally, in ρ^0 cells we observe indications of aberrant regulation of a key modulator of PKA activity, Ras2. These studies provide evidence of biologically relevant connections between nutrient metabolism and overall gene expression.

Acknowledgements

I would like to thank Dr. Michael Schultz for his continued enthusiasm for biochemistry in general and the research presented in this thesis. His approach to science has made my time in his lab productive and enjoyable. I would also like to thank Dr. Luis Schang and Dr. James Smiley for helpful discussions. Dr. Bob Wu, Darren Hockman, and Stacey Reinke are thanked for technical assistance and their contributions to experiments presented in this thesis. Finally I would like to thank members of the Schultz lab (Dr. Karen Robinson, Laura Minard, Brett Clelland, and Kathy Lin) as well as members of the Stuart lab (Dr. Sheetal Raithatha, Dr. James Decesare, and Matt Rawluk) for general scientific discussion.

Table of contents

Chapter	Page
1. Introduction	1
1.1 Acetylation of the nucleosome	2
1.2 Enzymatic control of histone acetylation	4
1.3 Terminology: targeted versus untargeted (global) histone acetylation	7
1.4 Targeted histone acetylation:	9
1.4.1 reading the genetic code	9
1.4.2 dynamics of histone post translational modifications	11
1.4.3 modification architecture of a transcribed gene	14
1.4.4 replication control	17
1.5 Untargeted histone acetylation and deacetylation <i>in vivo</i> : transcriptional regulation	20
1.6 How might untargeted KAT/HDAC activity affect gene-specific patterns of acetylation?	25
1.7 The biochemistry of untargeted histone acetylation and deacetylation: candidate enzymes	28
1.8 Are any heterotypic KAT or HDAC complexes specialized for untargeted modification of nucleosomes?	30
1.9 Physiological regulation of untargeted KATs and HDACs	33
1.10 Interaction of KATs and the chromatin assembly machinery during S-Phase	36

1.11	A system for studying physiological control of overall histone acetylation: glucose refeeding	38
1.12	Glucose: metabolism and cell signaling	40
1.13	Scope of the thesis	46
1.14	References	52
2.	Materials and Methods	62
2.1	Yeast strains	63
2.2	Yeast culture	64
2.3	Fractionation of SP cultures	66
2.4	Metabolic labeling	67
2.5	Drug treatment	68
2.6	Cell analysis and determination of glucose concentration of the culture medium	69
2.7	Fractionation of soluble histones from histones in chromatin	70
2.8	Protein isolation and immunoblotting	71
2.9	mRNA expression: quantitative real-time reverse transcriptase PCR	72
2.10	mRNA expression: Northern blotting	75
2.11	mRNA expression: microarray analysis	76
2.12	Profiling of cellular metabolites by ^1H -NMR spectroscopy	77
2.13	Enzymatic measurement of cellular acetate	79
2.14	References	85

3.	Glucose regulation of overall histone acetylation	87
3.1	Introduction	88
3.2	Results and Discussion	90
3.2.1	Glucose regulation of H3/H4 acetylation in SP cells	90
3.2.2	Glucose induction of H3/H4 acetylation in SP is a replication-independent phenomenon	92
3.3	Conclusions	97
3.4	References	105
4.	KAT and HDAC control of histone acetylation after glucose refeed	107
4.1	Introduction	108
4.2	Results and Discussion	112
4.2.1	Rpd3 partly controls steady-state H3/H4 acetylation in SP but does not have a major role in glucose induction of acetylation	112
4.2.2	Histone lysine acetylases Esa1 and Gcn5 mediate glucose induction of H3/H4 acetylation in SP	113
4.2.3	Glucose induction of histone acetylation by Esa1 and Gcn5 in SP is predominantly due to the untargeted activities of picNuA4 and SAGA	116
4.3	Conclusions	121
4.4	References	129

5.	Analysis of cell signaling and metabolic effects on histone acetylation	131
5.1	Introduction	132
5.2	Results and Discussion	138
5.2.1	Perturbation of two major glucose signaling modules does not interfere with glucose induction of histone acetylation in SP	138
5.2.2	A metabolic mechanism for glucose induction of global histone acetylation	142
5.2.3	Examining the sensitivity of overall histone acetylation to energy deprivation	148
5.3	Conclusions	151
5.4	References	161
6.	Mitochondrial defects impair the transcriptional response to removal of glucose	164
6.1	Introduction	165
6.1.1	Glucose-dependent signal transduction	165
6.1.2	Glucose regulation of transcription	169
6.2	Results and Discussion	174
6.2.1	p^0 cells lose overall acetylation of H4 in response to glucose removal	174
6.2.2	Microarray analysis of p^0 cells reveals a defect in derepression of glucose repressed genes after glucose removal	175

6.2.3 Examination of Snf1 signaling in ρ^0 cells: clues from analysis of transcriptional reprogramming	179
6.2.4 Biochemical analysis of activation of Snf1 signaling	181
6.2.5 Analysis of the PKA signaling pathway	183
6.2.6 What mechanism could account for inappropriate activation of the PKA pathway in ρ^0 cells?	187
6.3 Conclusions	191
6.4 References	201
7. Discussion	208
7.1 Glucose induction of overall H3/H4 acetylation does not depend upon replication coupled acetylation	209
7.2 Glucose induction of untargeted KAT activity	210
7.3 Chromatin interactions of KATs and HDACs that contribute to untargeted acetylation and deacetylation	213
7.4 Untargeted KAT activity is controlled by glycolytic metabolism	216
7.5 Acetylation of nonhistone proteins	221
7.6 Beyond yeast	222
7.7 Signaling defects displayed by cells lacking mtDNA	224
7.8 Future directions	228
7.8 References	231

List of tables

Table	Page
1.1 Known or potential targeting modules in selected yeast lysine acetylases (KATs) and histone deacetylases (HDACs).	48
2.1 Yeast strains used in this study.	80-84
6.1 Gene Ontology (GO) terms overrepresented in groups of genes that display a twofold or greater change in expression level.	195

List of Figures

Figure	Page
1.1 Nucleosome core particle with post translational modifications of the histones.	49
1.2 Fermentation/Glycolysis.	50
1.3 Ras and Gpr1/Gpa2 mediated glucose signaling.	51
3.1 Depletion of glucose and cell viability during the course of a refeeding experiment.	99
3.2 Overall histone acetylation on H3 and H4 is stimulated by glucose not amino acids during nutrient refeed.	100
3.3 Neither cell proliferation nor DNA replication are stimulated by refeeding glucose alone.	101
3.4 Glucose induces acetylation in G0 and non-quiescent cells, and glucose refeeding does not drive new synthesis of Cln2 or Clb5.	102
3.5 Overall acetylation in response to glucose refeed increases on nucleosomal histones even when protein translation is inhibited.	103
3.6 Glucose refeed produces a transcriptional response, but ongoing transcription is not required for histone re-acetylation.	104
4.1 Rpd3 protein expression in SP and effect of <i>RPD3</i> deletion on H4 acetylation.	123

List of Figures

Figure		Page
4.2	Identification of the KATs required for glucose induction of H4 acetylation in SP.	124
4.3	Identification of the KATs required for glucose induction of H3 acetylation in SP.	125
4.4	Identification of H4 KAT complex required for glucose induction of H4 acetylation in SP.	126
4.5	Identification of H3 KAT complex required for glucose induction of H3 acetylation in SP.	127
4.6	Analysis of involvement of targeting subunits of SAGA in glucose induction of H3 acetylation in SP.	128
5.1	Neither Ras- nor Gpr1/Gpa2-dependent PKA signalling is 'necessary and sufficient' for glucose induction of histone acetylation in SP cells.	153
5.2	Effect of rapamycin treatment on the response of SP cells to glucose refeeding.	154
5.3	Neither separate nor combined inhibition of PKA and Sch9 can block overall acetylation response to glucose.	155
5.4	Metabolite profiles in unfed and glucose-fed SP cells.	156

List of Figures

Figure	Page
5.5 Metabolic regulation of global histone acetylation by glucose.	157
5.6 Glucose induction of acetylation in SP requires full glycolytic processing of glucose.	158
5.7 Respiratory deficient cells display very low levels of histone acetylation in SP.	159
5.8 Overall acetylation on H4 decreases due to ATP depletion and increases with increased glucose supply.	160
6.1 Rapid loss of overall histone H4 acetylation after glucose removal in p^0 cells.	196
6.2 Activating phosphorylation of Snf1 occurs normally in p^{0f} cells but phosphorylation of the Snf1 substrate Mig1 is attenuated.	197
6.3 Hyperphosphorylation of Msn2 and Ume6, after glucose depletion, is blocked in p^0 cells.	198
6.4 Stationary phase p^0 cells fail to accumulate Acs1p.	199
6.5 Ume6 displays a mobility shift in p^0 <i>ras2Δ</i> cells but not wild type p^0 cells. During nutrient depletion Ras2p undergoes a mobility shift in p^+ cells but not p^0 cells.	200

Abbreviation/Term	Explanation
2-DDG	2-deoxy-D-glucose
ac	acetyl
ACS	A cetyl- C oenzyme A S ynthase
ADA	Ada2/Gcn5/Ada3 transcription activator complex; requires Ahc1 for structural integrity
CHD1	C hromatin organization modifier, H elicase, and D NNA-binding domains: component of SAGA and SLIK complexes binds K4 of histone H3 that is di- or tri-methylated
ChIP	C hromatin I mmunoprecipitation
CTD	C-Terminal Domain
EpcA	enhancer of polycomb
ESA1	E ssential S AS2-related A cetyltransferase 1 histone H4/H2A acetyltransferase
GCN5	G eneral C ontrol N onderepresable 5 histone H3/H2B acetyltransferase
GNAT	G CN5-related N - A cetyltransferases: Histone acetyltransferase family
GO	G ene O ntology
GTF	G eneral T ranscription F actor
HAT	H istone A cetyltransferase (now referred to as KAT)
HDAC	H istone D eacetylase
KAT	Lysine (K) Acetylase or A cetyltransferase
me	methyl
mtDNA	Mitochondrial DNA
MYST	M OZ, Y bf2/Sas3, S as2 and T ip60: Histone acetyltransferases family
1NM-PP1	4-Amino-1- <i>tert</i> -butyl-3-(1'-naphthylmethyl)pyrazolo[3,4-d]pyrimidine a cell permeable ATP analogue/Kinase inhibitor
NuA3	N ucleosomal histone H 3 A cetyltransferase complex

Abbreviation/Term

NuA4

Explanation

Nucleosomal histone **H4**

Acetyltransferase complex.

Houses Esa1 and Tra1. Involved in targeted acetylation

ORF

Open **R**eadin**G** **F**rame

PCAF

p300/**C**BP-associated **f**actor:

second GCN5 homologue found in vertebrates bearing approximately 73% identity to *Homo sapiens* GCN5 (Yang et al 1996)

PIC

Preinitiation **c**omplex

picNuA4

piccolo NuA4: Esa1 containing complex involved in untargeted histone acetylation

PKA

Protein **k**inase **A**

PKB

Protein **k**inase **B**

PKC

Protein **k**inase **C**

PTM

Post **T**ranslational **M**odification

PolII

RNA polymerase II

ρ^+ cell

Cell with mitochondrial DNA

ρ^0 cell

Cell without mitochondrial DNA

RSC

Remodeled the **S**tructure of **C**hromatin. Similar to the Swi/snf chromatin remodeling complex
SPT-ADA-GCN5 Acetyltransferase complex

SAGA

SAGA Altered **S**pt8 **A**bsent

(alternative name for SLIK)

S_C

Synthetic media without dextrose

SDC

Synthetic **D**extrose **C**omplete media also known as complete minimal media

SDS-PAGE

sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SLIK

SAGA Like complex

SNF

Sucrose **N**on-**F**ermentable

SP

Stationary **P**hase

STAGA

SPT3-TAF9-GCN5

Acetyltransferase: one of two GCN5/PCAF containing complexes equivalent to yeast SAGA
small ubiquitin-related modifier

SUMO

Abbreviation/Term**Explanation**

TFTC

TBP-Free-TAF Complex: one of two GCN5/PCAF containing complexes equivalent to yeast SAGA

Tip60

MYST family KAT

TOR

Target Of Rapamycin

TORC1

TOR Complex 1

TORC2

TOR Complex 2

TRA1

Component of SAGA and NuA4 acetyltransferase complexes required for interaction with transcriptional activators similar to human TRRAP

TBP

TATA Binding Protein

TRRAP

Transformation/transcription domain-associated protein

YPD

Yeast extract Peptone Dextrose rich growth media

Chapter 1: Introduction

Material in this chapter has been published:

Friis, R.M. and Schultz, M.C. (2009) Untargeted tail acetylation of histones in chromatin: lessons from yeast. *Biochem Cell Biol*, **87**, 107-116.

1.1 Acetylation of the nucleosome

Acetylation of histone protein components of nucleosome core particle, which consist of 146 bp of DNA wrapped around an octamer of histone proteins, exerts major effects upon functions that rely upon the DNA template in eukaryotic cells. Acetylation and deacetylation of lysine residues in the N-terminal tails of the histones (Figure 1.1), which extend from the surface of the nucleosome, have extensively characterized roles in transcriptional activation and repression, firing of replication origins, progression of replication, as well as in DNA damage repair (Aparicio et al., 2004; Knott et al., 2009; Kurdistani and Grunstein, 2003; van Attikum and Gasser, 2005; Vogelauer et al., 2002).

Numerous lysine residues within the histone H3/H4 tetramer and two H2A/H2B dimers which form a single histone protein octamer, are acetylated. Acetylation exerts its effects in various ways. As posited by the 'charge hypothesis', neutralization of the positive charge on lysine residues by acetylation of histones, may reduce the affinity of negatively charged DNA for histones (Grunstein, 1997). This loosening of nucleosomal structure could help non-histone proteins, such as transcription factors,

access the DNA template. In agreement with this, when adapted to provide single nucleosome resolution, genome wide chromatin immunoprecipitation (ChIP) studies demonstrate that, with the exception of acetylation at K16 of H4, the transcriptional effects of acetylation mainly depend upon the number of modified residues (Dion et al., 2005). Acetylation of certain residues can exert direct effects; acetylation of H4 K16, which is generally associated with transcriptionally active decondensed euchromatic regions, can itself prevent formation of higher order or condensed chromatin (Shogren-Knaak et al., 2006).

Acetylated lysine residues can also serve as docking sites for other protein factors involved in the regulation of DNA metabolism. Many of these factors contain one or more bromodomains which can interact with acetylated lysines. Bromodomain directed interactions with acetyl lysine residues have been characterized for chromatin remodeling complexes such as Swi/Snf and RSC, factors involved in initiation of transcription such as Bdf1 and Bdf2, and even in factors such as the lysine acetyltransferase (KAT) Gcn5, which mediates acetylation itself (Hassan et al., 2006; Hassan et al., 2002;

Kasten et al., 2004; Li and Shogren-Knaak, 2009; Matangkasombut and Buratowski, 2003).

1.2 Enzymatic control of histone acetylation

Families of enzymes which catalyze acetylation and deacetylation of histones are conserved from yeast to man. Histones are acetylated by KATs and acetyl groups can be removed by histone deacetylases (HDACs). There are three main classes of HDACs in *Saccharomyces cerevisiae*. Rpd3 is a Class I HDAC as are human HDACs 1-3 and 8 (Buggy et al., 2000). Yeast Hda1 and human HDACs 4-7 and 9 are Class II HDACs (Fischle et al., 2001; Zhou et al., 2000). The NAD dependent HDACs Sir2 and Hst1-4 (yeast) and HDACs SIRT1-7 (human) are Class III HDACs (Brachmann et al., 1995).

Major families of KATs are also represented in yeast. Yeast Gcn5, Hat1 and Elp3 as well as metazoan Gcn5 and PCAF (which is only found in vertebrates) are members of the Gcn5-related N-acetyltransferase (GNAT) family (Lee and Workman, 2007; Nagy and Tora, 2007). Additionally, several hundred eukaryotic genes have been classified as MYST (MOZ, Ybf2/Sas3, Sas2 and Tip60)

family KATs since notable sequence similarity between the acetyl-CoA binding domain of yeast Sas2 and Sas3 and human MOZ and Tip60 was recognized. Yeast Esa1, which bears resemblance to Tip60, also belongs to this group (Lafon et al., 2007).

The different KATs and HDACs display diverse, yet sometimes overlapping, substrate specificities. For example, Esa1 only acetylates sites on H2A, H4 and a H2A sequence variant Htz1, whereas Gcn5 acts on H3 and H2B. Other enzymes have considerable overlap in their sites of action. The most substantial overlap is seen with the HDACs Hos2 and Rpd3, which both act on residues in the amino terminal tails of H3 and H4. On H3 Rpd3 and Hos2 target the same sites but on H4 Hos2 acts at all four acetyl-lysines (K5, K8, K12, and K16), where Rpd3 only acts at three of them (K5, 8 and 12 but not K16). No two KATs or HDACs are known to act at exactly the same set of sites (Millar and Grunstein, 2006).

The substrate preferences of these enzymes are also affected by interactions with other proteins. Most KAT and HDAC enzymes are constituents of one or more multi protein complexes. The KAT complexes SAGA (Spt-Ada-Gcn5-

Acetyltransferase), SLIK (SAGA Like, also known as SALSA SAGA Altered Spt8 Absent), HAT-A2 and HATB3.1 all contain Gcn5; Esa1 is part of both NuA4 and piccolo NuA4 (picNuA4); Sas2 is found in the SAS complex and Sas3 is a component of NuA3. Similarly the HDAC Rpd3 is found in two complexes, RPD3S and RPD3L (Carrozza et al., 2005a; Carrozza et al., 2005b; Keogh et al., 2005).

The preference for interaction with free or nucleosomal histones can depend upon the complex in which an enzyme resides. Cases of this are evident for both Esa1 and Gcn5. In the context of NuA4, Esa1 acetylates free or nucleosomal histones with similar efficiency but Esa1 in picNuA4 acetylates nucleosomal histones in preference to free histones (Boudreault et al., 2003). In the context of SAGA, SLIK, or HAT-A2, Gcn5 acetylates nucleosomal histones in transcriptional circumstances; as part of HATB3.1 however, Gcn5 acetylates only free histones (Sklenar and Parthun, 2004). Specific residues which are acetylated by a KAT also depend upon complex composition. Grant *et al.* have noted that the ADA complex acetylates K14 and K18 of H3 while SAGA acetylated all four lysine residues they examined (Grant et al., 1999). Finally, cases have even

been seen where the actual histone component of an octamer that is acetylated by an enzyme is complex-dependent. In *Drosophila* Gcn5 is found in two complexes, STAGA and TFTC, which bear resemblance to yeast SAGA, and in a smaller third complex called ATAC (ADA two a containing). Similar to SAGA, STAGA and TFTC preferentially acetylate H3 in both free and nucleosomal histones. ATAC on the other hand acetylates free H3 and H4 histones and displays a preference for H4 in nucleosomes (Guelman et al., 2006). As will be seen in subsequent sections, these types of alterations in substrate specificity partially dictate the processes in which a KAT or HDAC is involved.

1.3 Terminology: targeted versus untargeted (global) histone acetylation

It is generally supposed that KATs and HDACs act on histone tails in the course of either untargeted or targeted encounters with chromatin. This idea was originally developed by groups studying histone acetylation in wild type and KAT/HDAC mutant strains of budding yeast using the method of chromatin immunoprecipitation (Krebs et al., 1999; Kuo et al.,

2000; Reid et al., 2000; Vogelauer et al., 2000). In these studies it was observed that KATs and HDACs have targeted effects on histone acetylation that reflect their recruitment to specific loci in the course of transcriptional induction or repression, as well as global (untargeted) effects that could not be attributed to chromatin recruitment associated with transcriptional regulation. Because untargeted encounters might occur anywhere along chromosomes, they have been said to control global histone acetylation (the terms 'untargeted' and 'global' acetylation are often used interchangeably in the literature). Subsequent research has clarified that targeting of KATs and HDACs occurs through their interaction with sequence specific DNA binding proteins or through interaction with a particular histone modification which is enriched at a particular loci during transcriptional processes (Brown et al., 2001; Carrozza et al., 2005a; Deckert and Struhl, 2002; Pray-Grant et al., 2005). The interaction of untargeted activities with chromatin do not depend upon sequence specific DNA binding proteins or post-translational marks established during transcription and therefore can potentially act upon any histone that provides a substrate for acetylation or deacetylation.

1.4 Targeted histone acetylation

In addition to the aforementioned effects upon substrate specificity, the components of both KAT and HDAC complexes regulate how these enzymes are recruited to chromatin. The regulatory role of a given KAT or HDAC is largely defined by the complex in which it resides. A wealth of molecular information is used to target KATs and HDACs activities. Elements within KAT/HDAC complexes allow them to be directed by DNA sequence and local modification state of histones. In addition to acetylation, histones are subject to mono- di- or tri- methylation, ubiquitination, SUMOlation, and phosphorylation. The role of DNA sequence and other histone modifications, in directing KAT or HDAC activity to specific loci during transcriptional regulation, will be discussed bellow.

1.4.1 Targeted histone acetylation: reading the genetic code

Association of transcriptional activators or repressors with specific sequences in promoters of protein coding genes is a key event in any transcriptional program. Sequence specific transcription factors are actually stable components of certain

HDAC complexes, such as RPD3L. Two sequence specific repressors, Ume6 and Ash1, are stable components of this complex (Carrozza et al., 2005a). Certain other complexes house components that can interact with numerous transcription factors, such as the three yeast KAT complexes, NuA4, SAGA, and SLIK. Tra1, a component of all of these complexes, directs interaction with transcriptional activators, thereby recruiting the complexes to promoters (Brown et al., 2001). The human homologue of Tra1, TRRAP (transformin/transcription domain-associated protein), which is required for c-Myc- and E1A-mediated oncogenic transformation, plays a similar role (McMahon et al., 1998). Originally isolated, via affinity purification, as a factor that interacts with the c-Myc N-terminus, TRRAP recruits STAGA to promoters via interaction with the transactivation domain of cMyc (Grant et al., 1998; Liu et al., 2003; McMahon et al., 1998; McMahon et al., 2000). Through these type of mechanisms, KAT or HDAC activities can be targeted to specific promoters to mediate local acetylation level.

1.4.2 Targeted histone acetylation: dynamics of histone post translational modifications

KAT and HDAC complexes also interact with post translational modifications (PTM) of histones, which were acquired during the process of transcriptional regulation. A dynamic interplay exists between many of the modifications such that addition or removal of a particular modification may depend upon prior modification of another residue. One of the first observed instances of one modification depending on another was at the *INO1* gene where phosphorylation of ser 10 on H3 by the AMPK homologue Snf1 is required for subsequent acetylation of H3 K14 by Gcn5 (Lo et al., 2001). The ability of one modification to block another is exemplified by the interactions between sumoylation and acetylation. Areas of chromatin which are enriched for one of these modifications show low levels of the other. Further, mutations which reduce overall levels of sumoylation increase overall acetylation level (Nathan et al., 2006). Tandem mass spectrometry was employed by Nathan *et al.* to determine the residues on histone H4 and H2B that are subject to sumoylation. Sumoylation occurs either on the same lysine residues as acetylation or lysine residues near them. As no lysine residue

was found simultaneously acetylated and sumoylated, these modifications are likely mutually exclusive or interfering (Nathan et al., 2006).

Two models have been proposed to explain the dynamic nature of chromatin modification and the regulatory significance of the PTMs. The histone code hypothesis suggested the modifications could be read sequentially or in combination to orchestrate downstream events (Jenuwein and Allis, 2001; Strahl and Allis, 2000). Schreiber and Bernstein (2002), later proposed the 'signaling network model' that suggested that the modification of histones was analogous to the modification of tyrosine receptor kinases, with modification serving as docking sites for adaptor proteins which control downstream events including subsequent modification (Schreiber and Bernstein, 2002). These models are not mutually exclusive, the main difference being that the signaling network model does not contend that the modifications can be read in combination.

These theories are supported by the presence of domains in factors involved in DNA and chromatin metabolism that bind modified and unmodified histones. In addition to bromodomain association with acetylated histones, chromodomains, WD40

domains, Tudor domains, MBT domains and the PHD finger have all been found to interact with methylated lysine residues (Aasland et al., 1995; Kim et al., 2006; Wysocka et al., 2006). The SANT domain (**S**wi3, **A**da2, **N**-Cor, and **T**FIIB), found in many corepressor complexes, including SMRT, CoREST and N-CoR, has been proposed to interact with unmodified histone tails. Interestingly this motif is also found in the Ada2 subunit of SAGA as well as in components of the Swi/Snf and RSC chromatin remodeling complexes; SANT domains may facilitate initial association of these complexes at unmodified loci. Recently, it has been discovered the both yeast and human 14-3-3 proteins, which are well known for binding to phosphorylated proteins, preferentially bind H3 tails that are doubly modified by phosphorylation at S10 and acetylation at K14 (Walter et al., 2008; Winter et al., 2008). Thus, whether a histone is decorated with numerous PTMs or unadorned, modules in specific factors can recognize these modifications (Table 1.1). The interactions between these modifications have the ability to establish domains, bearing a set of modifications, which provide an appropriate environment for the necessary DNA templated events at these loci.

1.4.3 Targeted acetylation: modification architecture of a transcribed gene

During transcription, a characteristic series of histone modifications are laid down which augment transcriptional control. A great deal of crosstalk between modifications has been observed. Interplay between ubiquitination and methylation and methylation with acetylation are evident. The di- or tri-methylation of H3 K4 by Set1 and methylation of H3 K79 by Dot1 require prior ubiquitination of H2B (Briggs et al., 2002). Conversely, the deubiquitination of H2B by Ubp8, a component of the SAGA complex, initiates events leading to recruitment of the Set2 methyltransferase and methylation of H3 K36 (Wyce et al., 2007). Both H3 K4 tri-methyl (me) and H3 K36 tri-me can interact with components of KAT and HDAC complexes. The chromodomain of Chd1, a component of SAGA and SLIK, targets these complexes through its interaction with H3 K4 tri-me (Pray-Grant et al., 2005). H3 K36 tri-me interacts with the PHD finger of Rco1 and the chromodomain of Eaf3 thereby recruiting the RPD3S complex in which Eaf3 and Rco1 are housed (Li et al., 2007). All of these interactions result in a characteristic pattern of modification at transcribed loci. (Pokholok et al., 2005)

characterized the genome-wide distribution of histone acetylation on H3 and H4, mono-, di- and tri- methylation of H3 K4, and tri-methylation of H3 K36. When normalized to histone occupancy level, a H3 and H4 acetylation peak is evident in the promoter regions of actively transcribed genes and declines within ORFs. Tri-me H3 K4 peaks at the 5'end of ORFs, di-me H3 K4 in the middle, and mono-me K4 at the 3'end. H3 K36 trimethylation was seen throughout ORFs with the highest levels at the 3'end .

This pattern is what would be expected based on the known sequential interaction between these modifications and complexes that recognize them. Transcriptional activator recruitment of SAGA/SLIK and NuA4 result in increased acetylation at a promoter. Gcn5 and Esa1 are generally recruited to promoters of active genes (Robert et al., 2004). Acetylation can further stabilize SAGA interaction in the area, via interaction with the bromodomain of Gcn5, and facilitate chromatin remodeling through recruitment of bromodomain containing remodeling complexes (Hassan et al., 2006; Hassan et al., 2002; Kasten et al., 2004; Li and Shogren-Knaak, 2009). Acetylation and remodeling activities facilitate association of general

transcription factors (GTFs) with the DNA, preinitiation complex (PIC) assembly, and recruitment of RNA Polymerase II (PolII) (Boeger et al., 2004; Deckert and Struhl, 2001; Reinke and Horz, 2003).

The ubiquitin-conjugating enzyme Rad6 and the E3 ubiquitin ligase Bre1 are recruited to the promoter, allowing for ubiquitination of H2B. Subsequently the PAF elongation complex facilitates their association with PolII, allowing these factors to act throughout the ORF (Wood et al., 2003; Xiao et al., 2005). The PolII C-Terminal Domain (CTD) is phosphorylated by TFIIF at the 5' end of genes, this phosphorylation recruits Set1 in a PAF-dependent manner allowing for tri-methylation of H3 K4 in the 5' region of transcribed ORFs (Ng et al., 2003). As PolII transcribes through an ORF, its CTD becomes phosphorylated at Ser-2 by Ctk1, resulting in recruitment of Set2 and trimethylation of K36 towards the 3' region of a gene (Xiao et al., 2003). H3 K4 methylation at the 5' end of a gene can further promote transcriptional activity by facilitating recruitment of SAGA/SLIK, thereby promoting maintenance of acetylation. Interestingly, this recruitment might also facilitate the deubiquitination of H2B by the SAGA component Ubp8.

Deubiquitination of H2B is required for recruitment of Ctk1 leading to phosphorylation of the PolII CTD at ser-2 and H3 K36 trimethylation (Wyce et al., 2007). Recruitment of RPD3S to H3 methylated at K36 leads to the decreased acetylation levels found within transcribed ORFs (Carrozza et al., 2005b; Keogh et al., 2005).

1.4.4 Targeted histone acetylation: replication control

There is a wealth of data consistent with the idea that chromatin acetylation affects the function of replication origins. Origin selection, activity, and frequency and timing of activation, all seem to be influenced by the level of acetylation of the nucleosomes that surround origins (reviewed in (Norio, 2006; Zhou et al., 2005). For example, genetic manipulations such as deletion of a major HDAC (Rpd3), or artificial targeting of an H3 KAT (Gnc5) to origins, strongly affect the timing of origin firing in budding yeast (Aparicio et al., 2004; Vogelauer et al., 2002).

Recent evidence suggests that Rpd3 exerts control over timing of replication origin firing mainly in the context of the RPD3L complex. Knott *et al.* analyzed replication origin firing

profiles for mutants which either disrupted RPD3S activity (*eaf3Δ*, *rco1Δ*, or *set2Δ*) or RPD3L (*dep1Δ*, or *cit6Δ*). Mutants in which RPD3L was disrupted, displayed replication origin firing profiles similar to those of *rpd3Δ* cells. Disruption of RPD3S had a much less pronounced effect upon origin firing, with these mutants presenting profiles that resembled those of wild type cells more closely. Overall more than 100 origins are regulated by Rpd3 in the context of RPD3L, where only 6 seem to be regulated by RPD3S (Knott et al., 2009).

RPD3L has an established role in targeted deacetylation at promoters. Previously Aparicio *et al.* attempted to assess the role of untargeted histone acetylation by deleting *UME6* and *TUP1*. Within RPD3L, Ume6 is responsible for Rpd3 recruitment to URS1 sites in the upstream activating region of some genes. Tup1 is a component of a transcriptional co-repressor known to recruit Rpd3 to specific *cis*-acting sequences. The fact that deletion of *UME6* or *TUP1* did not affect origin firing was taken to support the hypothesis that Rpd3 affects origin function by an untargeted mechanism (Aparicio et al., 2004). Knott *et al.* reexamined this idea, as many factors are implicated in targeting of Rpd3. In addition to Ume6, the sequence specific repressor

Ash1 is a stable component of RPD3L (Carrozza et al., 2005a). Analysis of the replication profiles of *ume6Δ* or *ash1Δ* cells suggested that neither Ume6 nor Ash1 contribute significantly to Rpd3-mediated control of replication origin firing (Knott et al., 2009). Comparison of replication origins affected by RPD3L with previously published data which characterized the genome wide effects on acetylation caused by *RPD3* deletion, the transcriptional profile of *rpd3Δ* cells, the transgenic regions bound by Rpd3, and ChIP chip data detailing binding sites for 203 transcription factors, elucidated how RPD3L might control replication firing (Knott et al., 2009). Replication origins that are affected by RPD3L disruption tend to be near regions bound by Rpd3 and genes regulated by Rpd3. These regions show increased histone acetylation in the absence of Rpd3 and binding of particular transcription factors (Knott et al., 2009). Together, these results suggest that many factors may contribute to the targeting of RPD3L, each of which contributes to Rpd3-mediated effects upon timing of origin firing.

1.5 Untargeted histone acetylation and deacetylation *in vivo*: transcriptional regulation

The pioneering work suggesting the existence of untargeted mechanisms for chromatin acetylation and deacetylation (above) has been supported by the results of more recent studies in yeast of the genome-wide distribution of KATs and HDACs, acetylated histones, and components of the basal transcriptional machinery. In combination with the results obtained from gene expression profiling of KAT and HDAC mutants, this work has strongly supported the notion that untargeted effects on chromatin acetylation are important in transcriptional regulation (Millar and Grunstein, 2006).

The precise function of untargeted chromatin acetylation and deacetylation in transcriptional regulation has long been a matter of speculation. Early on it was proposed that untargeted activities: 1) create a default underacetylated state that decreases basal transcription, and 2) promote the rapid turnover of acetyl groups that returns transcribed genes to a baseline acetylation state (Kuo et al., 2000; Reid et al., 2000; Vogelauer et al., 2000). Deletion of HDAC *RPD3* or *HDA1* results in a

general increase in H3K9 and H4K12 acetylation over a 4.5 kb region encompassing *PHO5* and two adjacent open reading frames (ORFs). These increases in acetylation are associated with increased transcription of *PHO5* under conditions that would normally repress its expression (Vogelauer et al., 2000).

Deleting H3 lysine acetylase *GCN5* in *rpd3Δ* cells prevents increases in *PHO5* transcription seen in the absence of Rpd3 (Vogelauer et al., 2000). These findings support the idea that the interplay between the untargeted activities of KATs and HDACs can establish basal transcription rates. The latter proposal was also supported by the work of Vogelauer *et al.* (2000). Expression of *PHO5* is induced in low-phosphate medium and repressed in high phosphate medium. Both *hda1Δ* and *hda1Δ rpd3Δ* cells, which displayed higher than wild type global histone acetylation levels, also displayed slower down-regulation of *PHO5* after transfer from inducing low-phosphate conditions to repressive high-phosphate conditions (Vogelauer et al., 2000).

Further evidence in favor of the hypothesis that untargeted KAT/HDAC activities promote rapid turnover of acetyl groups at promoters has been provided by Katan-Khaykovich and Struhl (2002). These studies used yeast strains engineered to express

one of two chimeric transcription factors, TetR-VP16 or TetR-Ume6. These factors respectively targeted KATs or the Rpd3 HDAC to *tet* operator sequences which were introduced in the upstream control region of chromosomal *HIS3* gene derivatives. The TetR chimeras could be rapidly dissociated from *tet* operator sequences by adding the tetracycline analog doxycycline (Dox) to the culture medium. It was found by ChIP that H3/H4 acetylation increased about 2-fold when Rpd3 targeting to the reporter gene was disrupted, and decreased about 2.5-fold when KAT targeting was disrupted (Katan-Khaykovich and Struhl, 2002). These changes occurred within 15 minutes of Dox treatment suggesting a high rate of constitutive turnover of acetylation in the amino-terminal tails of the histones (in accord with previous work – see (Waterborg, 2000; Waterborg, 2001). This turnover is likely due to untargeted enzymatic activities, since the upstream control region of the reporter gene lacked binding sites for endogenous transcription factors that might recruit KATs and HDACs. An alternative possibility, which we consider less likely, is that turnover of acetylation occurs by histone replacement. This possibility is suggested by recent evidence that histone replacement occurs continuously at

promoters independently of transcription and replication (Dion et al., 2007; Jamaï et al., 2007; Kim et al., 2007; Linger and Tyler, 2006; Rufiange et al., 2007). Thus, H3/H4 acetylation at *HIS3* in the presence of TetR-VP16 could be due to the activity of targeted KATs and the deposition of histones that had been acetylated in solution. The targeted contribution to acetylation would be lost under conditions that result in TetR-VP16 dissociation from the promoter, and acetylation would decline to a lower steady state level. This new level would be set by the activity of KATs directed towards soluble histones (see discussion in (Govind et al., 2007) for a similar argument applied to Gcn4-regulated genes). The time resolution of currently available methods for measuring histone replacement is low compared to the time resolution of the TetR/Dox system, therefore it is not known if untargeted histone acetylation and histone turnover occur at the same rate at *HIS3*. Therefore, the extent to which untargeted KATs and HDACs account for the results obtained using TetR/Dox-dependent control of KAT/HDAC localization remains somewhat uncertain.

A possible role for untargeted KAT activity in transcriptional regulation was uncovered in a recent analysis focused on the

conserved KAT Gcn5 (Imoberdorf et al., 2006). This study exploited an episomal *lacZ* reporter gene controlled by a model promoter (*xPHO5*) in which the upstream control region of *PHO5* was engineered to contain a single binding site for RFX, a transcriptionally inactive DNA binding protein. This system allows for transcriptional induction without activator recruitment of KATs.

Under non-inducing conditions the steady state level of H3K9 acetylation at *xPHO5* depends on Gcn5. Induction of *xPHO5* by direct recruitment of a TFIIB-RFX fusion protein occurred without Gcn5 recruitment or a change in histone acetylation, and was unaffected by *GCN5* deletion. Gcn5 however was required for transcription when the TATA box was mutated and when noncovalent interactions between TFIIB-myc and RFX-max fusion proteins were used to recruit TFIIB to the promoter. These findings support the notion that untargeted Gcn5 sets the level of acetylation at *xPHO5* to a point that is permissive for induction driven by weak recruitment of the basal transcription machinery. They also suggest that the strength of interaction between a particular activator and its target in the transcriptional machinery might determine the extent to which

induction of natural promoters depends on untargeted acetylation.

1.6 How might untargeted KAT/HDAC activity affect gene-specific patterns of acetylation?

We have outlined several functions of untargeted activities that contribute to optimal programming of transcription. In much of the genome these functions are executed in the context of chromatin that is concurrently acted on by a plethora of targeted activities. How is it that the 'random' effects of untargeted activities are tolerated in regions of the genome where targeted enzymes must act? This is a particularly important question in the context of recent evidence that targeted activities must establish or maintain patterns of acetylation that are necessary for correct execution of transcriptional programs.

The patterns of chromatin acetylation associated with genes have been revealed by single nucleosome resolution mapping of histone modifications (Liu et al., 2005). The mapping results suggest general roles for acetylation in transcription which are compatible with the presence of untargeted acetylation. It

appears that two general and simple patterns of modifications are associated with genes (Liu et al., 2005). A domain surrounding the transcriptional start site, that seems to occur regardless of transcription level, contains nucleosomes that are hypo-acetylated at H2AK7, H2BK18, H3K8, and H4 K8 and K16. The second notable set of modifications clusters to the 5' regions of ORFs, where there is enrichment of acetylation on H3K9 and K18, H4K5 and K12, and H2AK7. These acetylations occur in a gradient across ORFs from 5' to 3', with highest levels in the 5' region. Since different levels of transcription (indirectly assessed by Pol II occupancy) were not associated with distinct patterns of acetylation, Liu and colleagues concluded that they do not have a code-like function (Liu et al., 2005). Therefore, we propose that the simple architecture of gene-associated modifications will be fairly resilient, from a functional viewpoint, to small variations in acetylation due to untargeted activities. To put it another way, untargeted KAT/HDAC activity can be tolerated in regions associated with genes because there is no strict code that would be fatally corrupted by untargeted enzyme activity.

While Liu et al. (2005) found no evidence that different combinations of modifications result in different levels of

transcription, they did observe that the level of enrichment of the modifications generally found in the 5' regions of genes was correlated with transcription. This finding is consistent with the results obtained by analysis of the global effects on transcription of single or combinatorial mutations of acetyltable lysines in the tail of H4 (Dion et al., 2005). These results revealed that acetylation of H4 residues K5, K8, and K12 works in an additive fashion, with greater levels of acetylation associated with higher transcriptional capacity. We suggest that the function of untargeted enzymes is to establish a global level of acetylation that, under inducing conditions, is permissive for binding of activators in the upstream control regions of target genes. The set point of global acetylation might be fine-tuned so that, generally speaking, recruitment of activators to less preferred targets (for example, genes whose upstream control region includes an imperfect match to the consensus binding site) is disfavoured. Subsequent to activator binding, acetylation is increased by targeted enzymes to a level that somehow facilitates transcription. During active transcription untargeted activities will have little effect on local acetylation due to higher local concentrations of targeted activities.

1.7 The biochemistry of untargeted histone acetylation and deacetylation: candidate enzymes

Early on it was proposed that untargeted effects on chromatin acetylation result from the activity of the soluble nuclear forms of KATs and HDACs acting unselectively on nucleosomes (Kuo and Allis, 1998). In other words, untargeted KATs and HDACs were predicted to interact with nucleosomal histones in a rather promiscuous manner, independently of the features of chromatin that differ from one place in the genome to another.

Examinations of the *in vitro* substrate specificity of Gcn5 and Esa1 have revealed that heterotypic complexes containing these KATs readily acetylate free histones and nucleosomes. In contrast, recombinant versions of these KATs were able to acetylate free histones but not nucleosomes (Allard et al., 1999; Boudreault et al., 2003; Grant et al., 1997). Although one subsequent study has shown that rGcn5 is capable of acetylating nucleosomes, when the buffer salt concentrations were optimized, these KATs, and the other individual yeast proteins that have histone-directed KAT activity, exist in the cell primarily

as components of heterotypic protein complexes (Lee and Workman, 2007; Tse et al., 1998). These soluble complexes are likely to be responsible for virtually all untargeted acetylation in the amino-terminal tails of the histones.

Most HDAC enzymes in yeast, like the KATs, function in complex with other proteins. Untargeted deacetylation at most lysine residues in the amino-terminal tails of the histones is therefore reasonably expected to result from the activity of heterotypic HDAC complexes. This expectation is consistent with biochemical studies of the HDA1 complex, which have shown that its catalytic subunit is inactive against nucleosomes in the absence of the Hda2 and Hda3 subunits of the complex (Carmen et al., 1999). The exception is deacetylation of H2BK11. This reaction is performed by the NAD^+ -dependent Hos3 enzyme, which is active as a homodimer (Carmen et al., 1999). Since deletion of *HOS3* blocks overall deacetylation of H2BK11 in apoptotic yeast cells, it is possible that Hos3 is an untargeted H2BK11 HDAC (Ahn et al., 2006). Based on the considerations outlined above, it appears likely that every KAT and HDAC in the nucleus might be capable of untargeted activity towards nucleosomal histones.

1.8 Are any heterotypic KAT or HDAC complexes specialized for untargeted modification of nucleosomes?

At least on first principles there is no need to invoke the existence of enzymes specialized for untargeted acetylation/deacetylation, since targeted KATs and HDACs likely have some untargeted encounters with chromatin (Howe et al., 2001). It does appear, however, that one KAT in yeast is specialized for untargeted chromatin acetylation. The catalytic subunit of that KAT is Esa1, an H2A/H4 acetylase. Esa1 exists in two protein complexes, NuA4 and Piccolo NuA4 (picNuA4; (Boudreault et al., 2003). NuA4, the larger of the complexes, is formed of at least 13 proteins. PicNuA4 is comprised of just four polypeptides - Esa1, Epl1, Yng2, and Eaf6 - which are also found in NuA4 (Boudreault et al., 2003; Doyon et al., 2006). While both NuA4 and picNuA4 acetylate free and nucleosomal H2A and H4, only picNuA has a preference for nucleosomal histones.

Three features of picNuA4 seem to contribute to its preference for nucleosomal histones. These are the enhancer of polycomb (EpcA) homology region in Epl1, the N-terminal 165 amino acids of Yng2, and the chromodomain of Esa1 (Selleck et

al., 2005). The chromodomain is well characterized as being specialized for methyl-lysine binding (Taverna et al., 2007). Surprisingly, although required for preference of nucleosomal over free histones, the chromodomain in Esa1 does not predispose picNuA4 to acetylate methylated in preference to unmethylated nucleosomes. And it appears to be dispensable for picNuA4 binding to nucleosomes, even though it can bind unacetylated H3 tails (Jacobs et al., 2001; Selleck et al., 2005). How then might the chromodomain in picNuA4 contribute to its preference for nucleosomal histone substrates? One possibility is that it facilitates acetylation after nucleosome binding by altering histone tail contacts with DNA (Selleck et al., 2005).

What picNuA4 does not contain suggests as much about its function as what it does contain. Most importantly, Côté and colleagues (Boudreault et al., 2003) found that picNuA4 lacks the Tra1 protein, which is important for promoter targeting of NuA4 (Nourani et al., 2004; Vignali et al., 2000). Since they could not detect any physical interaction between picNuA4 and three well-characterized transcriptional activators, Boudreault *et al.* proposed that picNuA4 is specialized for untargeted H4 acetylation in the cell.

Human cells contain a H4 KAT complex, HBO1, which has similar properties to picNuA4 (Doyon et al., 2006); its catalytic HBO1 subunit is in the same KAT family as Esa1, it includes Yng2-related and Eaf6-related proteins (ING4 or ING5 and hEAF6 respectively), and it prefers nucleosomal over free H4 as a substrate (its target sites in H4 are K5, 8 and 12). These findings, and the fact that knockdown of HBO1 protein causes a specific decline in overall H4 K5, 8, 12 acetylation (Doyon et al., 2006), are consistent with the idea that untargeted chromatin acetylation by HBO1 is important for cellular physiology in human. It is not yet clear what that untargeted function might be. One possibility, since knockdown of HBO1 complex subunits HBO1 or ING5 causes a replication defect in tissue culture cells (Doyon et al., 2006), is that untargeted acetylation by HBO1 is important for replication control. On the other hand, since interactions with replication proteins likely direct HBO1 complexes to replicating DNA (Doyon et al., 2006 and references therein), the untargeted activity of HBO1 might be more important for transcriptional regulation. The latter hypothesis remains to be addressed experimentally.

It is not yet known if any HDAC in yeast or other organisms preferentially functions by an untargeted mechanism. That is not to say that HDAC inhibition, either by pharmacological or genetic interventions, is without an effect on overall acetylation. Indeed, there are now numerous examples in higher organisms of effects on overall acetylation resulting from HDAC inhibition that are associated with cellular phenotypes of potential medical importance (Knutson et al., 2008; Li et al., 2006; Meshorer and Misteli, 2006). The point, however, is that phenotypes arising from general inhibition of HDAC activities are not necessarily due to loss of untargeted activity: it is equally plausible that they are due (for example) to defects in targeted HDAC activity at multiple loci or at a restricted number of loci from which HDAC activity spreads. More work is needed to determine if any HDAC can preferentially act in an untargeted fashion on nucleosomal histones.

1.9 Physiological regulation of untargeted KATs and HDACs

Physiological signals no doubt control how much untargeted KAT and HDAC activity is present in the nucleus. This control is

likely exerted by multiple mechanisms. The simplest of these probably dictate the relative nucleoplasmic abundance of KATs versus HDACs. Two mechanisms for this regulation immediately spring to mind. One could control the total expression level of the KATs/HDACs. picNuA4 is one example of an untargeted H4 KAT that might be controlled at the level of protein expression; it is more readily obtained from cells experiencing nutrient limitation and from cells arrested in G2/M by nocodazole treatment (Boudreault et al., 2003). Such regulation might involve signalling that impinges on the catalytic subunit of a KAT/HDAC complex, as reported for the NAD^+ -dependent HDAC Hst3 (Thaminy et al., 2007). Differential regulation of the activities of relevant nuclear export and import pathways could also control the nucleoplasmic concentration of KATs/HDACs. Such a mechanism has been reported for Hst2, another NAD^+ -dependent HDAC of yeast (Wilson et al., 2006).

The intrinsic activity of untargeted enzymes might be subject to physiological regulation by such mechanisms as transient association with activators or inhibitors, or post-translational modification of enzyme subunits. The latter possibility is raised by evidence that the activity of KATs/HDACs

can be modulated by phosphorylation of their catalytic subunits (recent examples are described in Huang and Chen, 2005; McGee et al., 2008; Pluemsampant et al., 2008). Since the targeting efficiency of one broad-specificity KAT, the Gnc5-related p300 protein in human, depends partly on its phosphorylation state (Huang and Chen, 2005), we speculate that the pool of enzyme available for untargeted activity is also controlled by enzyme modification state.

Finally it is also interesting to consider the possibility that untargeted enzyme activity is regulated by the availability of enzyme co-substrates, acetyl-coenzyme A (acetyl-CoA) in the case of KATs and NAD^+ in the case of some HDACs. In particular, there is compelling evidence that maintenance of a high steady-state level of overall acetylation in actively proliferating yeast cells requires ongoing activity of the acetyl-CoA synthase enzymes Acs1 and Acs2 (Takahashi et al., 2006). Perhaps then the availability of acetyl-CoA for use by untargeted enzymes is a focal point for the regulation of global histone acetylation. Clearly there are many interesting questions that remain concerning the molecular mechanisms that underlie physiological regulation of untargeted KATs and HDACs.

1.10 Interaction of KATs and the chromatin assembly machinery during S-Phase

Acetylation of various residues within histones appears to play an important role in the deposition of newly synthesized histones. This acetylation and deposition is most active during, but is not limited to, S-phase. DNA replication is coupled to increased synthesis of histones, allowing for chromatinization of the nascent genome. These newly synthesized histones are acetylated by several KATs (classically referred to as B type HATs (histone acetyltransferases)); thus overall acetylation levels increase in concert with bulk histone levels during S-phase (Adkins et al., 2007; Rocha and Verreault, 2008). In this process, acetylation of both K5 and K12 on histone H4 is well conserved from yeast to mammals. Although not required for assembly, acetylation of H4 K5 and K12 becomes required if the N-terminal tail of H3 is deleted (Ma et al., 1998; Sobel et al., 1995). Functional redundancy of the H3 and H4 tails during assembly is not unlikely. Histones H3 and H4 are assembled into a nucleosome as a unit (Kornberg, 1977; Thomas and Kornberg, 1975a; Thomas and Kornberg, 1975b).

Acetylation of the N-terminal tail of newly synthesized H3 is also conserved although the specific sites of acetylation H3 are not (Sobel et al., 1995). The role of H3 tail acetylation in assembly has received far less attention than the role of H4 tail acetylation.

Two sites of acetylation in the globular domain of histones, one in H4 and one in H3, are associated with replication and are found in yeast and man. Histone H4 is acetylated at K91 (Ye et al., 2005). Mass spectrometric analysis of H4 molecules which co-purified with the B-type HAT complex (Hat1 and Hat2 in complex with the histone chaperone Hif1) provided the first evidence that H4 K91 is acetylated. Histone H4 K91 maps in a region in the nucleosome where the H3/H4 tetramer makes contact with the H2A/H2B dimer (Ye et al., 2005). Histone H3 is acetylated at K56 by Rtt109 in yeast , CBP (Nejire) in flies, and p300 and CBP in humans (Das et al., 2009). The side chain of K56 is surface-exposed and is proximal to the point of DNA entry to and exit from the nucleosome. As such it is postulated to affect DNA affinity for the nucleosome. Mutation of K91 of H4 or K56 of H3 result in phenotypes indicative of chromatin assembly defects and genomic instability. All of these findings point

towards histone acetylation playing an influential role in chromatin assembly (Chen et al., 2008; Das et al., 2009; Li et al., 2008; Ye et al., 2005).

1.11 A system for studying physiological control of overall histone acetylation: Glucose refeeding

The overall histone acetylation state results from acetylation of newly synthesized histones, together with the level of acetylation left on nucleosomes by KAT and HDAC activities functioning in targeted and untargeted acetylation or deacetylation. Physiological resetting of overall histone acetylation uncoupled from replication has been described in mammalian cells. For example, H3 K9 and H4 acetylation are induced prior to S phase in mitogenically stimulated B and T cells (Baxter et al., 2004; Taplick et al., 1998), H4 acetylation is induced one day after the onset of embryonic stem cell differentiation (Meshorer et al., 2006), H3/H4 acetylation is induced in cells of the hippocampus and cortex during neuronal rewiring (Fischer et al., 2007), and H3 K9 acetylation is induced in the course of epigenetic reprogramming in the germ line (Hajkova et al., 2008). Despite the abundant evidence that

overall histone acetylation is subject to physiological regulation in non-replicating cells, little is known about the functional significance of changes in overall acetylation or the mechanisms by which changes are regulated.

We wished to develop a system which would allow us to manipulate overall acetylation under physiological conditions. For this purpose we chose stationary phase (SP) yeast as a model. In batch culture yeast grow and divide rapidly using carbon sources that can yield energy via fermentation, the glycolytic breakdown of sugar producing ethanol (Figure 1.2). When fermentable carbon sources have been depleted, yeast undergo a transcriptional and metabolic reprogramming known as the diauxic shift. This reprogramming facilitates respiratory metabolism of other carbon sources in the medium including the ethanol that was produced by fermentation. Once external carbon sources are depleted, cell growth and division halts. At this point yeast in the saturated culture establish a quiescent state in SP. Quiescent yeast cells arrest in an unbudded state with a G1 content of DNA and a thickened cell wall (de Nobel et al., 2000). Rates of transcription in quiescent yeast are 3 to 5

fold lower than those of cycling cells, and chromosomes are condensed (Choder, 1991; Pinon, 1978; Schafer et al., 2008).

Previous studies have established that overall levels of histone acetylation decline as batch cultures of yeast reach saturation and enter SP (Ramaswamy et al., 2003; Sandmeier et al., 2002). When re-inoculated into nutrient replete medium, overall histone acetylation rebounds and cell proliferation resumes (as demonstrated in Chapter 2). Thus SP cultures provide a relatively synchronized population of G1-like cells in which rapid induction of overall acetylation can be achieved simply by introduction of nutrients. Our initial experiments demonstrate this reacetylation of histones is specifically a response to glucose. As much of cellular physiology is subject to control by signaling pathways, it is tempting to speculate that changes in overall acetylation resulting from glucose refeed might be controlled by pathways that are implicated in mediation of the response to glucose.

1.12 Glucose: metabolism and cell signaling

In unicellular organisms such as the yeast *S. cerevisiae*, extracellular nutrients are sensed and elicit cellular responses

analogous to those that result from hormonal stimuli in metazoans (Dechant and Peter, 2008). Cell signaling events influence transcriptional reprogramming and nutrient metabolism to allow the optimal response to presence or absence of glucose in the local environment.

Glucose, the preferred carbon source of *Saccharomyces*, is catabolized in glycolysis yielding two molecules of ATP and two molecules of pyruvate (Figure 1.2). Due to the organization of the yeast metabolic and transcriptional apparatus in the presence of glucose, pyruvate is not processed in the TCA cycle but is decarboxylated by pyruvate decarboxylase, forming acetaldehyde that can either be reduced by alcohol dehydrogenase to form ethanol, or oxidized by aldehyde dehydrogenase to form acetate (Figure 1.2). Metabolism of glucose yields energy and intermediates required for anabolic processes.

The yeast homologue of the AMP sensitive kinase Snf1 controls the expression of a number of genes which are required for metabolism of alternative carbon sources. To a large extent the strong preference for glucose displayed by *Saccharomyces* is maintained by a network of interactions which keep Snf1 in an

inactive state when glucose is present. The set of genes regulated by inhibition of Snf1 activity represents only a small fraction of the genes whose expression changes in response to glucose (Zaman et al., 2009).

The addition of glucose to post diauxic cells or cells that are growing on a respiratory carbon source results in massive transcriptional reprogramming. The glucose refeeding of post diauxic cells conducted by Liko *et al.* (Liko et al., 2007) resulted in a greater than two fold induction of 1355 genes. Similarly, when glucose was added to yeast cultures grown using glycerol as the carbon source, more than 40% of genes displayed a greater than two fold change in expression level (Wang et al., 2004). Approximately 90% of genes whose expression levels change in response to glucose display similar changes in expression level in response to either activation of Protein Kinase A (PKA) or overexpression of the AGC family kinase (homologous to protein kinases A, G and C) Sch9 (Zaman et al., 2009). Under normal physiological conditions however, PKA activation seems to play a more dominant role in control of the response to glucose than Sch9.

Intracellular cAMP level increases in response to glucose, and this activates PKA. Inactive PKA is found as a heterotetramer composed of two catalytic subunits (encoded by *TPK1*, *TPK2* and *TPK3*) in yeast and two regulatory subunits (encoded by *BCY1*). Binding of cAMP to the regulatory subunits results in their dissociation from the catalytic subunits, thereby activating the kinase. Two parallel pathways have been implicated in mediating the cAMP response to glucose (Figure 1.3). One of these is controlled by the small GTPases Ras1 and Ras2. Activation of the Ras proteins via GTP loading is facilitated by the GTP exchange factor (GEF) Cdc25 (Broek et al., 1987). Deactivation is mediated by the intrinsic Ras GTPase activity which can be stimulated by the GTPase activating proteins (GAPs) Ira1 and Ira2 (Tanaka et al., 1989; Tanaka et al., 1990a; Tanaka et al., 1990b). Although the precise mechanism remains unknown, feeding glucose to starved cells results in increased Ras GTP levels. Active Ras GTP can bind to the adenylate cyclase, Cyc1 (otherwise known as Cdc35), and activate it resulting in production of cAMP (Broek et al., 1985; Toda et al., 1985). The second pathway is controlled by Gpr1, a G-protein coupled receptor homologue, and its interacting GTP binding

protein partner Gpa2. Gpa2 appears to activate adenylate cyclase in response to binding of glucose or sucrose to Gpr1 (Lemaire et al., 2004). Indirect evidence gleaned from analysis of mutant forms of Gpr1 suggests that glucose and sucrose are Gpr1 ligands (Lemaire et al., 2004). Moreover, recombinant Gpa2 is capable of binding Cyr1 when loaded with GTP but not GDP (Peeters et al., 2006). Consistent with the Ras and Gpr1/Gpa2 pathways being the principal regulators of the PKA pathway, deletion of *RAS2* is synthetic lethal with deletion of either *GPR1* or *GPA2* and this lethality can be overcome by deletion of *PDE2*, a gene which encodes a phosphodiesterase responsible for breakdown of cAMP (Kubler et al., 1997; Xue et al., 1998).

The Broach lab have demonstrated that 90% of the transcriptional response elicited by addition of glucose to cells grown on glycerol can be recapitulated by expression of either the constitutively active Ras2^{G19V} or the Gpa2^{Q300L} allele from an inducible promoter (Wang et al., 2004). All of these effects are mediated through activation of PKA (Wang et al., 2004; Zaman et al., 2009). The Ras proteins may mediate a greater portion of the response to glucose than Gpr1/Gpa2. The magnitude of

transcriptional changes initiated by expression of Gpa2^{Q300L} is only about half of that seen for glucose addition or expression of Ras2^{G19V}, which are of very similar magnitude (Wang et al., 2004). Furthermore, deletion of *GPR1* has limited or no effect on the strength of glucose induced transcriptional changes. Induction of a dominant negative version of Ras2 however, was capable of reducing the PKA regulated transcriptional response to glucose feeding to one quarter of that in wild type cells (Zaman et al., 2009). This is similar to the effect of specific inhibition of PKA during glucose refeed (Zaman et al., 2009).

As eluded to above, Sch9, which bears homology to the mammalian kinases Akt/PKB and S6 kinase, plays a minor role in comparison to PKA in mediation of the response to glucose. Sch9 was originally cloned in an overexpression screen looking for proteins that could suppress the temperature sensitivity of *cdc25^{ts}* mutants (Toda et al., 1988). The same study revealed that Sch9 overexpression could repress defects resulting from various mutations in the Ras PKA pathway, including deletion of all three genes encoding the catalytic subunit of PKA (Toda et al., 1988). Although overexpression of Sch9 exerts the same transcriptional effects as activation of PKA, inhibiting Sch9 during

glucose refeed does not diminish the glucose response (Zaman et al., 2009). While this suggests that Sch9 has either no role or a minimal one in the glucose response, Sch9 may be able to play a compensatory role in the absence of PKA. As stated above, 90% of glucose regulated genes are similarly regulated by activation of PKA. Inhibition of PKA activity, during glucose refeed, reduces the magnitude of the transcriptional response of these genes to one quarter of what is seen in response to glucose feeding without prior inhibition of PKA. If however, both PKA and Sch9 are simultaneously inhibited, this set of genes does not display any transcriptional response (Zaman et al., 2009).

1.13 Scope of the thesis

The control of cellular responses to glucose has been highly studied, and glucose refeeding provides a model in which large changes in overall histone acetylation can be induced. An attractive initial hypothesis was that activation of PKA upon glucose refeeding triggers a PKA-mediated transcriptional response that is responsible for induction of overall histone acetylation by glucose. This hypothesis and others are tested in

the following chapters. Chapter 2 presents the materials and methods used for the experimental analyses described in chapters 3-6. Chapter 3 describes experiments which establish that induction of acetylation in fresh media is specifically a response to glucose, and explores the hypothesis that increases in overall histone acetylation in response to glucose refeeding could be a result of cell cycle reentry and mechanisms coupled to replication. Chapter 4 examines the KATs and HDACs that mediate the glucose response, and the complexes in which the enzymes controlling glucose driven acetylation reside are characterized. Analysis of the complexes involved shed light upon the contribution of targeted and untargeted acetylation to overall acetylation. Chapter 5 highlights the examination of the mechanism by which glucose induces overall histone acetylation. Chapter 6 discusses aberrations that occur in Snf1 and PKA signaling in cells with mitochondrial defects. These abnormalities were revealed during studies examining the effect of reduced overall histone acetylation on establishment of a new transcriptional program. Chapter 7 discusses the findings presented in the thesis as a whole.

Table 1.1 Known or potential targeting modules in selected yeast lysine acetylases (KATs) and histone deacetylases (HDACs).

Enzyme Subunits	Enzyme activity <i>in vitro</i> ^a	Protein Domains with targeting activity	Target ^b
Hos3 homodimer	KAT	DNA	Upstream of <i>MET10</i> <i>in vitro</i> (1)
Hos3 Taf4	As above	Unknown	Taf4 subunit of TFIID (2)
SAGA complex			
Gcn5	KAT	Bromo	Kac
Ada2		SANT	unacetylated histone tails
Spt7		Bromo	Kac
Sgf29		Tudor	Kme?
NuA4 complex			
Esa1	KAT	Chromo	Kme
Eaf1		SANT	unacetylated histone tails
Eaf2		SANT	unacetylated histone tails
Eaf3		Chromo	Kme
Yng2		PHD	Kme
picNuA4 complex			
Esa1	KAT	Chromo	Kme
Yng2		PHD	Kme
NuA3 complex			
Sas3	KAT		
Nto1		PHD	Kme
Yng1		PHD	Kme
RPD3L complex			
Rpd3	HDAC		
Pho23		PHD	Kme
Cti6		PHD	Kme
Ume6		DNA	TSGGCGGCTAW (3)
Ash1		DNA	YTGAT (4)
RPD3S complex			
Rpd3	HDAC		
Rco1		PHD	Kme
Eaf3		Chromo	Kme
Set3C			
Set3		PHD, SET	Kme
Snt1		SANT	unacetylated histone tails
Hos2	HDAC		
Hst1	HDAC		

Note: ^aEnzyme activities have been variously measured using 3 substrates: histone N-terminal tail peptides, free histones, and nucleosomal histones. Histone and site specificity are given only for assays against nucleosomal histones. ^bThe histones and residues and residues recognized by the various domains are summarized in detail by (Taverna et al., 2007).

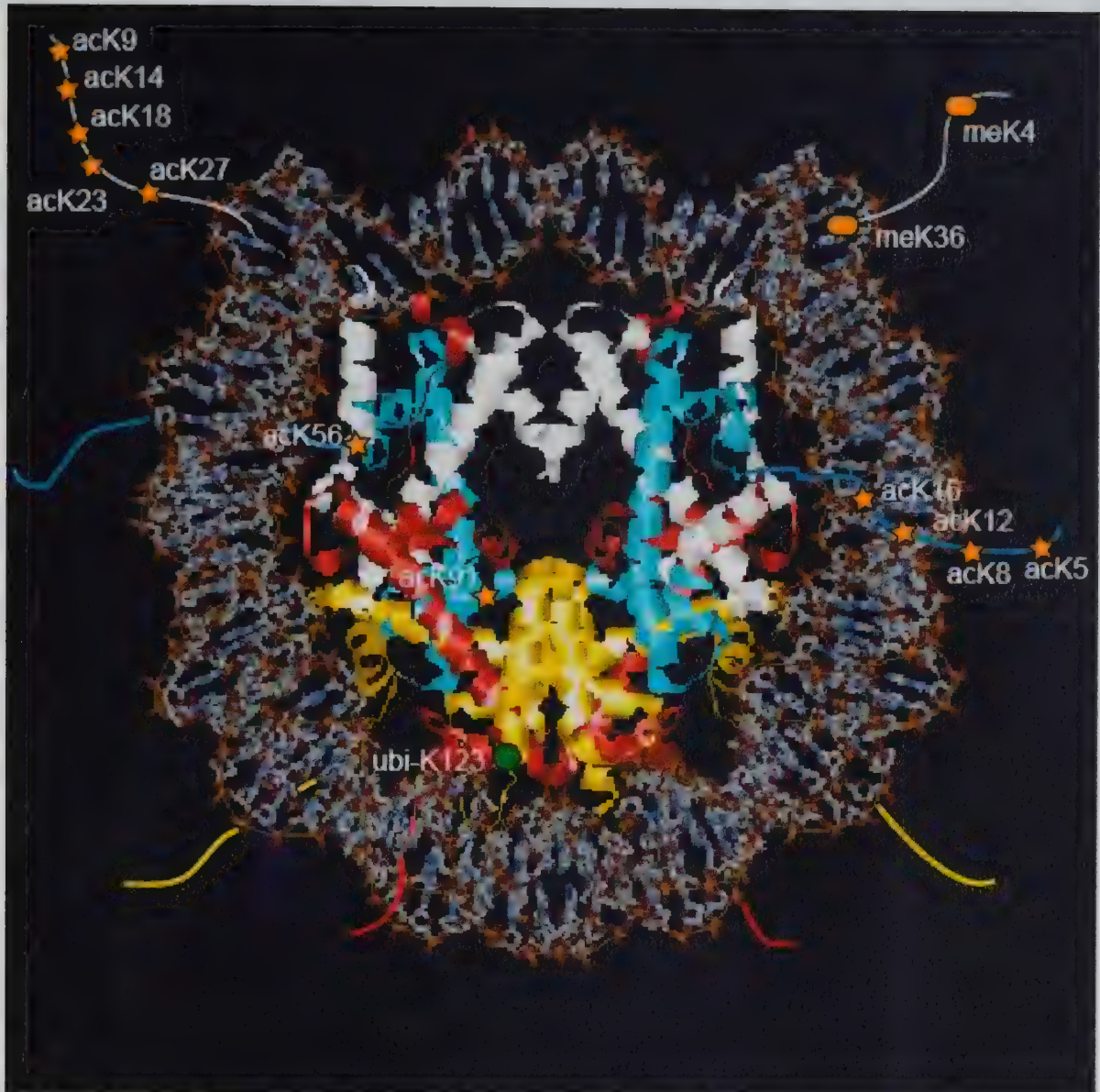


Figure 1.1 Nucleosome core particle with drawn representation of histone tails and positions of post translational modifications of the histones.

Yeast nucleosome with 146 base pairs of DNA. Histone H3 (white); Histone H4 (cyan); Histone H2A (red); Histone H2B (yellow). Acetylated lysine residues (acK) are highlighted with stars, methylated lysines (meK) with rounded rectangles, and ubiquitinated lysine (ubi-K) with a hexagon. Image adapted from (White et al., 2001) using RasWin Molecular graphics version 2.7.5 from <http://www.rasmol.org/>

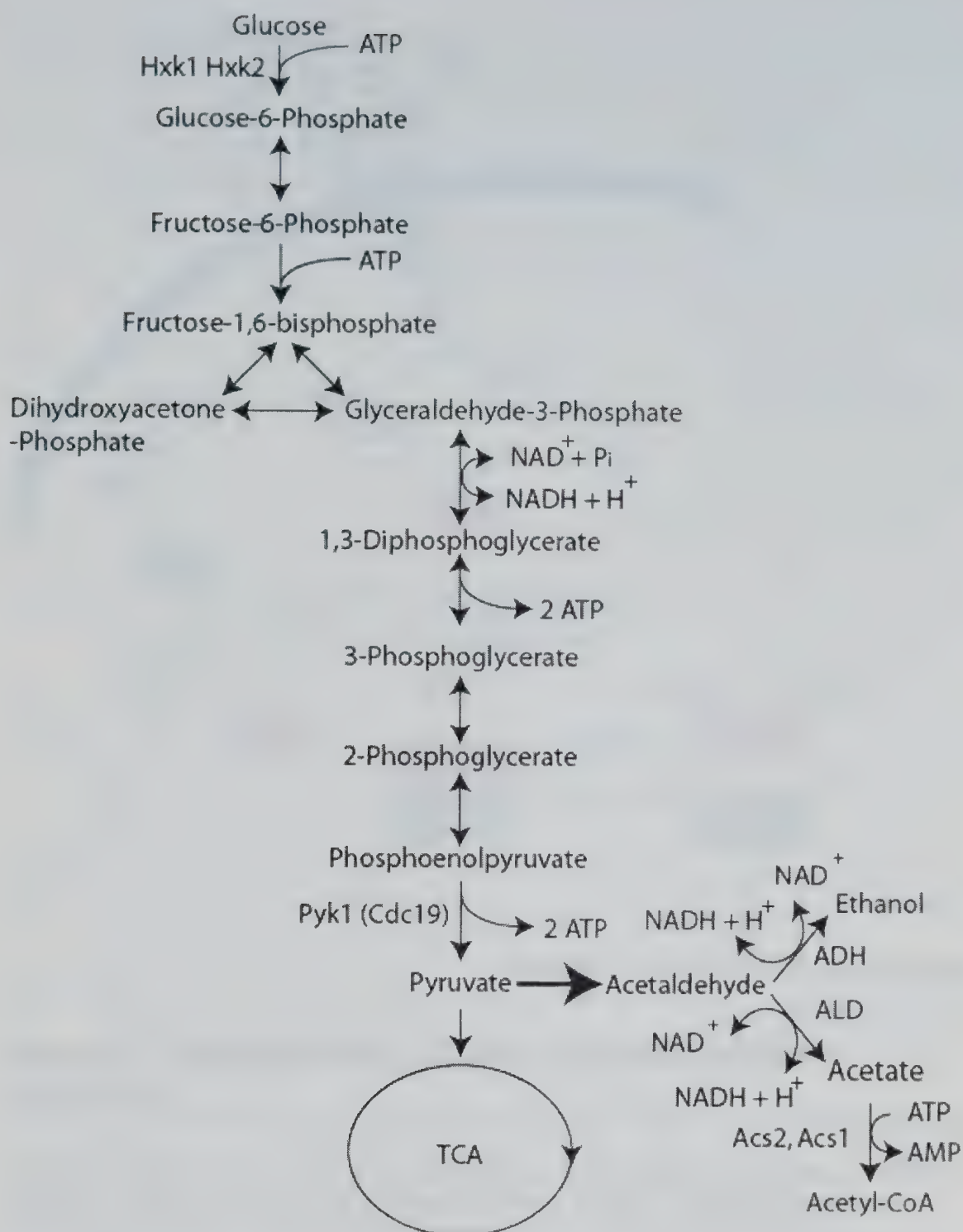


Figure 1.2 Fermentation/Glycolysis.

Glucose is metabolized to pyruvate through glycolysis. In *Saccharomyces cerevisiae*, in the presence of glucose, pyruvate is primarily decarboxylated to acetaldehyde which can in turn be reduced to ethanol by the alcohol dehydrogenases (ADH), or oxidized to form acetate by the aldehyde dehydrogenases (ALD).

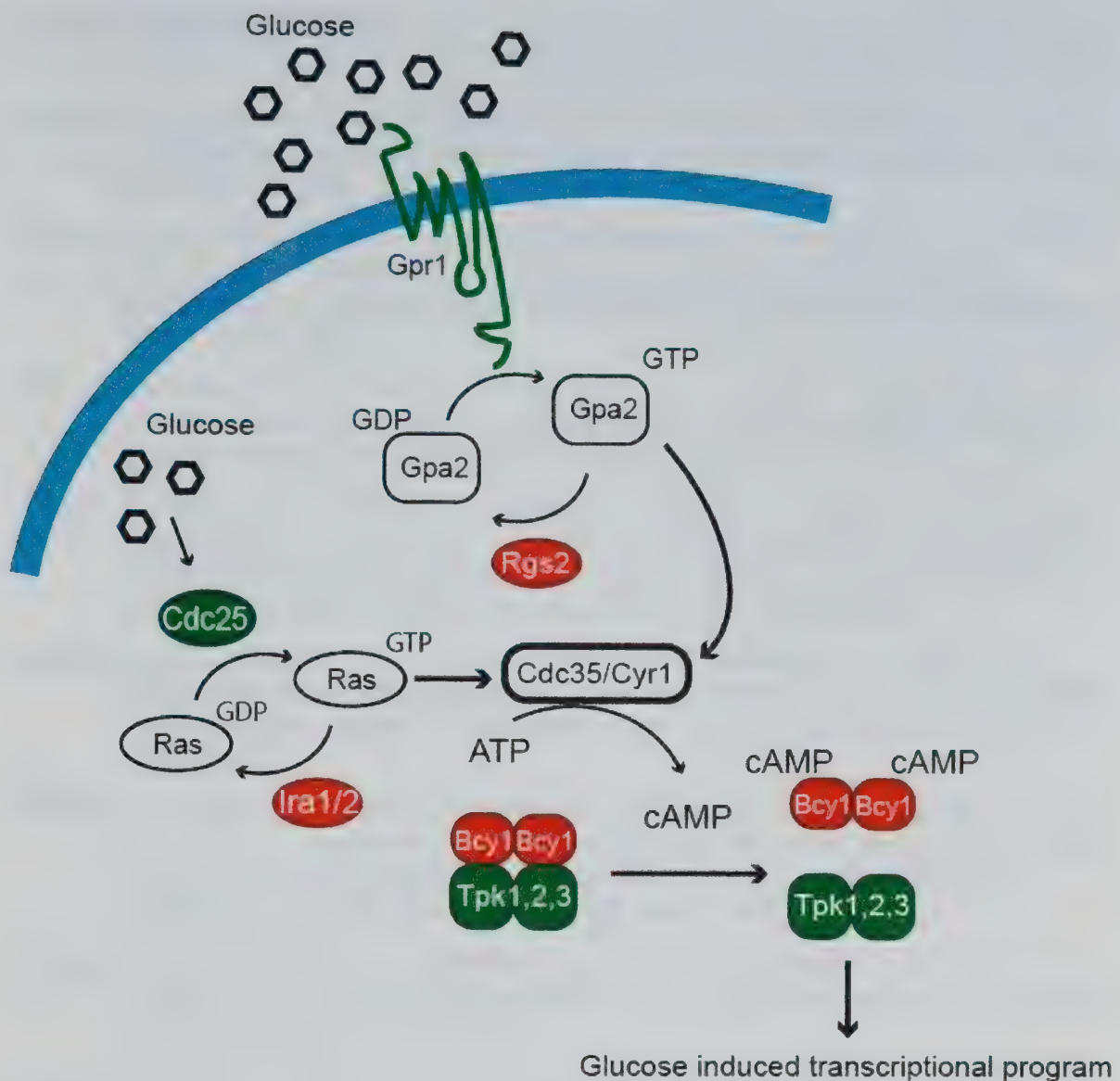


Figure 1.3 Ras and Gpr1/Gpa2 mediated glucose signaling.

Activation of the GTP binding proteins Ras1, Ras2 (**Ras**) and **Gpa2**, via GTP loading, results in activation of the adenylyl cyclase, **Cdc35/Cyr1**, and production of cAMP. cAMP binds the regulatory subunit of PKA, **Bcy1**, resulting in release of the catalytic PKA subunit, encoded redundantly by **TPK1**, **TPK2**, and **TPK3**. Activation of the Ras proteins is facilitated by the Ras GEF, **Cdc25**, and antagonized by the Ras GAPs, **Ira1/2**. Gpa1 is activated by the G-protein coupled receptor homologue, **Gpr1**, and can be inactivated by the GAP, **Rgs2**.

1.14 References

- Aasland, R., Gibson, T.J. and Stewart, A.F. (1995) The PHD finger: implications for chromatin-mediated transcriptional regulation. *Trends Biochem Sci*, **20**, 56-59.
- Adkins, M.W., Carson, J.J., English, C.M., Ramey, C.J. and Tyler, J.K. (2007) The histone chaperone anti-silencing function 1 stimulates the acetylation of newly synthesized histone H3 in S-phase. *J Biol Chem*, **282**, 1334-1340.
- Ahn, S.H., Diaz, R.L., Grunstein, M. and Allis, C.D. (2006) Histone H2B deacetylation at lysine 11 is required for yeast apoptosis induced by phosphorylation of H2B at serine 10. *Mol Cell*, **24**, 211-220.
- Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L., Workman, J.L. and Cote, J. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *Embo J*, **18**, 5108-5119.
- Aparicio, J.G., Viggiani, C.J., Gibson, D.G. and Aparicio, O.M. (2004) The Rpd3-Sin3 histone deacetylase regulates replication timing and enables intra-S origin control in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **24**, 4769-4780.
- Baxter, J., Sauer, S., Peters, A., John, R., Williams, R., Caparros, M.L., Arney, K., Otte, A., Jenuwein, T., Merckenschlager, M. and Fisher, A.G. (2004) Histone hypomethylation is an indicator of epigenetic plasticity in quiescent lymphocytes. *Embo J*, **23**, 4462-4472.
- Boeger, H., Griesenbeck, J., Strattan, J.S. and Kornberg, R.D. (2004) Removal of promoter nucleosomes by disassembly rather than sliding in vivo. *Mol Cell*, **14**, 667-673.
- Boudreault, A.A., Cronier, D., Selleck, W., Lacoste, N., Utley, R.T., Allard, S., Savard, J., Lane, W.S., Tan, S. and Cote, J. (2003) Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev*, **17**, 1415-1428.
- Brachmann, C.B., Sherman, J.M., Devine, S.E., Cameron, E.E., Pillus, L. and Boeke, J.D. (1995) The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. *Genes Dev*, **9**, 2888-2902.
- Briggs, S.D., Xiao, T., Sun, Z.W., Caldwell, J.A., Shabanowitz, J., Hunt, D.F., Allis, C.D. and Strahl, B.D. (2002) Gene silencing: trans-histone regulatory pathway in chromatin. *Nature*, **418**, 498.
- Broek, D., Samiy, N., Fasano, O., Fujiyama, A., Tamanoi, F., Northup, J. and Wigler, M. (1985) Differential activation of yeast adenylate cyclase by wild-type and mutant RAS proteins. *Cell*, **41**, 763-769.
- Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S. and Wigler, M. (1987) The *S. cerevisiae* CDC25 gene product regulates the RAS/adenylyl cyclase pathway. *Cell*, **48**, 789-799.

- Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S. and Workman, J.L. (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science*, **292**, 2333-2337.
- Buggy, J.J., Sideris, M.L., Mak, P., Lorimer, D.D., McIntosh, B. and Clark, J.M. (2000) Cloning and characterization of a novel human histone deacetylase, HDAC8. *Biochem J*, **350 Pt 1**, 199-205.
- Carmen, A.A., Griffin, P.R., Calaycay, J.R., Rundlett, S.E., Suka, Y. and Grunstein, M. (1999) Yeast HOS3 forms a novel trichostatin A-insensitive homodimer with intrinsic histone deacetylase activity. *Proc Natl Acad Sci U S A*, **96**, 12356-12361.
- Carrozza, M.J., Florens, L., Swanson, S.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005a) Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. *Biochim Biophys Acta*, **1731**, 77-87; discussion 75-76.
- Carrozza, M.J., Li, B., Florens, L., Suganuma, T., Swanson, S.K., Lee, K.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005b) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell*, **123**, 581-592.
- Chen, C.C., Carson, J.J., Feser, J., Tamburini, B., Zabaronick, S., Linger, J. and Tyler, J.K. (2008) Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell*, **134**, 231-243.
- Choder, M. (1991) A general topoisomerase I-dependent transcriptional repression in the stationary phase in yeast. *Genes Dev*, **5**, 2315-2326.
- Das, C., Lucia, M.S., Hansen, K.C. and Tyler, J.K. (2009) CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature*, **459**, 113-117.
- de Nobel, H., Ruiz, C., Martin, H., Morris, W., Brul, S., Molina, M. and Klis, F.M. (2000) Cell wall perturbation in yeast results in dual phosphorylation of the Slt2/Mpk1 MAP kinase and in an Slt2-mediated increase in FKS2-lacZ expression, glucanase resistance and thermotolerance. *Microbiology*, **146 (Pt 9)**, 2121-2132.
- Dechant, R. and Peter, M. (2008) Nutrient signals driving cell growth. *Curr Opin Cell Biol*, **20**, 678-687.
- Deckert, J. and Struhl, K. (2001) Histone acetylation at promoters is differentially affected by specific activators and repressors. *Mol Cell Biol*, **21**, 2726-2735.
- Deckert, J. and Struhl, K. (2002) Targeted recruitment of Rpd3 histone deacetylase represses transcription by inhibiting recruitment of Swi/Snf, SAGA, and TATA binding protein. *Mol Cell Biol*, **22**, 6458-6470.
- Dion, M.F., Altschuler, S.J., Wu, L.F. and Rando, O.J. (2005) Genomic characterization reveals a simple histone H4 acetylation code. *Proc Natl Acad Sci U S A*, **102**, 5501-5506.

- Dion, M.F., Kaplan, T., Kim, M., Buratowski, S., Friedman, N. and Rando, O.J. (2007) Dynamics of replication-independent histone turnover in budding yeast. *Science*, **315**, 1405-1408.
- Doyon, Y., Cayrou, C., Ullah, M., Landry, A.J., Cote, V., Selleck, W., Lane, W.S., Tan, S., Yang, X.J. and Cote, J. (2006) ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Mol Cell*, **21**, 51-64.
- Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M. and Tsai, L.H. (2007) Recovery of learning and memory is associated with chromatin remodelling. *Nature*, **447**, 178-182.
- Fischle, W., Dequiedt, F., Fillion, M., Hendzel, M.J., Voelter, W. and Verdin, E. (2001) Human HDAC7 histone deacetylase activity is associated with HDAC3 in vivo. *J Biol Chem*, **276**, 35826-35835.
- Govind, C.K., Zhang, F., Qiu, H., Hofmeyer, K. and Hinnebusch, A.G. (2007) Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. *Mol Cell*, **25**, 31-42.
- Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev*, **11**, 1640-1650.
- Grant, P.A., Eberharter, A., John, S., Cook, R.G., Turner, B.M. and Workman, J.L. (1999) Expanded lysine acetylation specificity of Gcn5 in native complexes. *J Biol Chem*, **274**, 5895-5900.
- Grant, P.A., Schieltz, D., Pray-Grant, M.G., Yates, J.R., 3rd and Workman, J.L. (1998) The ATM-related cofactor Tra1 is a component of the purified SAGA complex. *Mol Cell*, **2**, 863-867.
- Grunstein, M. (1997) Histone acetylation in chromatin structure and transcription. *Nature*, **389**, 349-352.
- Guelman, S., Suganuma, T., Florens, L., Swanson, S.K., Kiesecker, C.L., Kusch, T., Anderson, S., Yates, J.R., 3rd, Washburn, M.P., Abmayr, S.M. and Workman, J.L. (2006) Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel dAda2A/dGcn5-containing histone acetyltransferase complex in *Drosophila*. *Mol Cell Biol*, **26**, 871-882.
- Hajkova, P., Ancelin, K., Waldmann, T., Lacoste, N., Lange, U.C., Cesari, F., Lee, C., Almouzni, G., Schneider, R. and Surani, M.A. (2008) Chromatin dynamics during epigenetic reprogramming in the mouse germ line. *Nature*, **452**, 877-881.
- Hassan, A.H., Awad, S. and Prochasson, P. (2006) The Swi2/Snf2 bromodomain is required for the displacement of SAGA and the octamer transfer of SAGA-acetylated nucleosomes. *J Biol Chem*, **281**, 18126-18134.

- Hassan, A.H., Prochasson, P., Neely, K.E., Galasinski, S.C., Chandy, M., Carrozza, M.J. and Workman, J.L. (2002) Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. *Cell*, **111**, 369-379.
- Howe, L., Auston, D., Grant, P., John, S., Cook, R.G., Workman, J.L. and Pillus, L. (2001) Histone H3 specific acetyltransferases are essential for cell cycle progression. *Genes Dev*, **15**, 3144-3154.
- Huang, W.C. and Chen, C.C. (2005) Akt phosphorylation of p300 at Ser-1834 is essential for its histone acetyltransferase and transcriptional activity. *Mol Cell Biol*, **25**, 6592-6602.
- Imoberdorf, R.M., Topalidou, I. and Strubin, M. (2006) A role for gcn5-mediated global histone acetylation in transcriptional regulation. *Mol Cell Biol*, **26**, 1610-1616.
- Jacobs, S.A., Taverna, S.D., Zhang, Y., Briggs, S.D., Li, J., Eissenberg, J.C., Allis, C.D. and Khorasanizadeh, S. (2001) Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *Embo J*, **20**, 5232-5241.
- Jamai, A., Imoberdorf, R.M. and Strubin, M. (2007) Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol Cell*, **25**, 345-355.
- Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science*, **293**, 1074-1080.
- Kasten, M., Szerlong, H., Erdjument-Bromage, H., Tempst, P., Werner, M. and Cairns, B.R. (2004) Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. *Embo J*, **23**, 1348-1359.
- Katan-Khaykovich, Y. and Struhl, K. (2002) Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes Dev*, **16**, 743-752.
- Keogh, M.C., Kurdistani, S.K., Morris, S.A., Ahn, S.H., Podolny, V., Collins, S.R., Schuldiner, M., Chin, K., Punna, T., Thompson, N.J., Boone, C., Emili, A., Weissman, J.S., Hughes, T.R., Strahl, B.D., Grunstein, M., Greenblatt, J.F., Buratowski, S. and Krogan, N.J. (2005) Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell*, **123**, 593-605.
- Kim, H.J., Seol, J.H., Han, J.W., Youn, H.D. and Cho, E.J. (2007) Histone chaperones regulate histone exchange during transcription. *Embo J*, **26**, 4467-4474.
- Kim, J., Daniel, J., Espejo, A., Lake, A., Krishna, M., Xia, L., Zhang, Y. and Bedford, M.T. (2006) Tudor, MBT and chromo domains gauge the degree of lysine methylation. *EMBO Rep*, **7**, 397-403.
- Knott, S.R., Viggiani, C.J., Tavare, S. and Aparicio, O.M. (2009) Genome-wide replication profiles indicate an expansive role for Rpd3L in regulating replication initiation timing or efficiency, and reveal genomic loci of

- Rpd3 function in *Saccharomyces cerevisiae*. *Genes Dev*, **23**, 1077-1090.
- Knutson, S.K., Chyla, B.J., Amann, J.M., Bhaskara, S., Huppert, S.S. and Hiebert, S.W. (2008) Liver-specific deletion of histone deacetylase 3 disrupts metabolic transcriptional networks. *Embo J*, **27**, 1017-1028.
- Kornberg, R.D. (1977) Structure of chromatin. *Annu Rev Biochem*, **46**, 931-954.
- Krebs, J.E., Kuo, M.H., Allis, C.D. and Peterson, C.L. (1999) Cell cycle-regulated histone acetylation required for expression of the yeast HO gene. *Genes Dev*, **13**, 1412-1421.
- Kubler, E., Mosch, H.U., Rupp, S. and Lisanti, M.P. (1997) Gpa2p, a G-protein alpha-subunit, regulates growth and pseudohyphal development in *Saccharomyces cerevisiae* via a cAMP-dependent mechanism. *J Biol Chem*, **272**, 20321-20323.
- Kuo, M.H. and Allis, C.D. (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays*, **20**, 615-626.
- Kuo, M.H., vom Baur, E., Struhl, K. and Allis, C.D. (2000) Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. *Mol Cell*, **6**, 1309-1320.
- Kurdistani, S.K. and Grunstein, M. (2003) Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol*, **4**, 276-284.
- Lafon, A., Chang, C.S., Scott, E.M., Jacobson, S.J. and Pillus, L. (2007) MYST opportunities for growth control: yeast genes illuminate human cancer gene functions. *Oncogene*, **26**, 5373-5384.
- Lee, K.K. and Workman, J.L. (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol*, **8**, 284-295.
- Lemaire, K., Van de Velde, S., Van Dijck, P. and Thevelein, J.M. (2004) Glucose and sucrose act as agonist and mannose as antagonist ligands of the G protein-coupled receptor Gpr1 in the yeast *Saccharomyces cerevisiae*. *Mol Cell*, **16**, 293-299.
- Li, B., Gogol, M., Carey, M., Lee, D., Seidel, C. and Workman, J.L. (2007) Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. *Science*, **316**, 1050-1054.
- Li, Q., Zhou, H., Wurtele, H., Davies, B., Horazdovsky, B., Verreault, A. and Zhang, Z. (2008) Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell*, **134**, 244-255.
- Li, S. and Shogren-Knaak, M.A. (2009) The Gcn5 bromodomain of the SAGA complex facilitates cooperative and cross-tail acetylation of nucleosomes. *J Biol Chem*, **284**, 9411-9417.
- Li, Y., Kao, G.D., Garcia, B.A., Shabanowitz, J., Hunt, D.F., Qin, J., Phelan, C. and Lazar, M.A. (2006) A novel histone deacetylase pathway regulates mitosis by modulating Aurora B kinase activity. *Genes Dev*, **20**, 2566-2579.

- Liko, D., Slattery, M.G. and Heideman, W. (2007) Stb3 binds to ribosomal RNA processing element motifs that control transcriptional responses to growth in *Saccharomyces cerevisiae*. *J Biol Chem*, **282**, 26623-26628.
- Linger, J. and Tyler, J.K. (2006) Global replication-independent histone H4 exchange in budding yeast. *Eukaryot Cell*, **5**, 1780-1787.
- Liu, C.L., Kaplan, T., Kim, M., Buratowski, S., Schreiber, S.L., Friedman, N. and Rando, O.J. (2005) Single-nucleosome mapping of histone modifications in *S. cerevisiae*. *PLoS Biol*, **3**, e328.
- Liu, X., Tesfai, J., Evrard, Y.A., Dent, S.Y. and Martinez, E. (2003) c-Myc transformation domain recruits the human STAGA complex and requires TRRAP and GCN5 acetylase activity for transcription activation. *J Biol Chem*, **278**, 20405-20412.
- Lo, W.S., Duggan, L., Emre, N.C., Belotserkovskya, R., Lane, W.S., Shiekhata, R. and Berger, S.L. (2001) Snf1--a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. *Science*, **293**, 1142-1146.
- Ma, X.J., Wu, J., Altheim, B.A., Schultz, M.C. and Grunstein, M. (1998) Deposition-related sites K5/K12 in histone H4 are not required for nucleosome deposition in yeast. *Proc Natl Acad Sci U S A*, **95**, 6693-6698.
- Matangkasombut, O. and Buratowski, S. (2003) Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. *Mol Cell*, **11**, 353-363.
- McMahon, S.B., Van Buskirk, H.A., Dugan, K.A., Copeland, T.D. and Cole, M.D. (1998) The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell*, **94**, 363-374.
- McMahon, S.B., Wood, M.A. and Cole, M.D. (2000) The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. *Mol Cell Biol*, **20**, 556-562.
- Meshorer, E. and Misteli, T. (2006) Chromatin in pluripotent embryonic stem cells and differentiation. *Nat Rev Mol Cell Biol*, **7**, 540-546.
- Meshorer, E., Yellajoshula, D., George, E., Scambler, P.J., Brown, D.T. and Misteli, T. (2006) Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev Cell*, **10**, 105-116.
- Millar, C.B. and Grunstein, M. (2006) Genome-wide patterns of histone modifications in yeast. *Nat Rev Mol Cell Biol*, **7**, 657-666.
- Nagy, Z. and Tora, L. (2007) Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene*, **26**, 5341-5357.
- Nathan, D., Ingvarsdottir, K., Sterner, D.E., Bylebyl, G.R., Dokmanovic, M., Dorsey, J.A., Whelan, K.A., Krsmanovic, M., Lane, W.S., Meluh, P.B., Johnson, E.S. and Berger, S.L. (2006) Histone sumoylation is a negative regulator in *Saccharomyces cerevisiae* and shows dynamic

- interplay with positive-acting histone modifications. *Genes Dev*, **20**, 966-976.
- Ng, H.H., Robert, F., Young, R.A. and Struhl, K. (2003) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell*, **11**, 709-719.
- Norio, P. (2006) DNA replication: the unbearable lightness of origins. *EMBO Rep*, **7**, 779-781.
- Nourani, A., Utley, R.T., Allard, S. and Cote, J. (2004) Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. *Embo J*, **23**, 2597-2607.
- Peeters, T., Louwet, W., Gelade, R., Nauwelaers, D., Thevelein, J.M. and Versele, M. (2006) Kelch-repeat proteins interacting with the Galpha protein Gpa2 bypass adenylate cyclase for direct regulation of protein kinase A in yeast. *Proc Natl Acad Sci U S A*, **103**, 13034-13039.
- Pinon, R. (1978) Folded chromosomes in non-cycling yeast cells: evidence for a characteristic g0 form. *Chromosoma*, **67**, 263-274.
- Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolzheimer, E., Zeitlinger, J., Lewitter, F., Gifford, D.K. and Young, R.A. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell*, **122**, 517-527.
- Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd and Grant, P.A. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature*, **433**, 434-438.
- Ramaswamy, V., Williams, J.S., Robinson, K.M., Sopko, R.L. and Schultz, M.C. (2003) Global control of histone modification by the anaphase-promoting complex. *Mol Cell Biol*, **23**, 9136-9149.
- Reid, J.L., Iyer, V.R., Brown, P.O. and Struhl, K. (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol Cell*, **6**, 1297-1307.
- Reinke, H. and Horz, W. (2003) Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. *Mol Cell*, **11**, 1599-1607.
- Robert, F., Pokholok, D.K., Hannett, N.M., Rinaldi, N.J., Chandy, M., Rolfe, A., Workman, J.L., Gifford, D.K. and Young, R.A. (2004) Global position and recruitment of HATs and HDACs in the yeast genome. *Mol Cell*, **16**, 199-209.
- Rocha, W. and Verreault, A. (2008) Clothing up DNA for all seasons: Histone chaperones and nucleosome assembly pathways. *FEBS Lett*, **582**, 1938-1949.
- Rufiange, A., Jacques, P.E., Bhat, W., Robert, F. and Nourani, A. (2007) Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell*, **27**, 393-405.

- Sandmeier, J.J., French, S., Osheim, Y., Cheung, W.L., Gallo, C.M., Beyer, A.L. and Smith, J.S. (2002) RPD3 is required for the inactivation of yeast ribosomal DNA genes in stationary phase. *Embo J*, **21**, 4959-4968.
- Schafer, G., McEvoy, C.R. and Patterson, H.G. (2008) The *Saccharomyces cerevisiae* linker histone Hho1p is essential for chromatin compaction in stationary phase and is displaced by transcription. *Proc Natl Acad Sci U S A*, **105**, 14838-14843.
- Schreiber, S.L. and Bernstein, B.E. (2002) Signaling network model of chromatin. *Cell*, **111**, 771-778.
- Selleck, W., Fortin, I., Sermwittayawong, D., Cote, J. and Tan, S. (2005) The *Saccharomyces cerevisiae* Piccolo NuA4 histone acetyltransferase complex requires the Enhancer of Polycomb A domain and chromodomain to acetylate nucleosomes. *Mol Cell Biol*, **25**, 5535-5542.
- Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R. and Peterson, C.L. (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science*, **311**, 844-847.
- Sklenar, A.R. and Parthun, M.R. (2004) Characterization of yeast histone H3-specific type B histone acetyltransferases identifies an ADA2-independent Gcn5p activity. *BMC Biochem*, **5**, 11.
- Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T. and Allis, C.D. (1995) Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc Natl Acad Sci U S A*, **92**, 1237-1241.
- Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature*, **403**, 41-45.
- Takahashi, H., McCaffery, J.M., Irizarry, R.A. and Boeke, J.D. (2006) Nucleocytosolic acetyl-coenzyme A synthetase is required for histone acetylation and global transcription. *Mol Cell*, **23**, 207-217.
- Tanaka, K., Matsumoto, K. and Toh, E.A. (1989) IRA1, an inhibitory regulator of the RAS-cyclic AMP pathway in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **9**, 757-768.
- Tanaka, K., Nakafuku, M., Satoh, T., Marshall, M.S., Gibbs, J.B., Matsumoto, K., Kaziro, Y. and Toh-e, A. (1990a) *S. cerevisiae* genes IRA1 and IRA2 encode proteins that may be functionally equivalent to mammalian ras GTPase activating protein. *Cell*, **60**, 803-807.
- Tanaka, K., Nakafuku, M., Tamanoi, F., Kaziro, Y., Matsumoto, K. and Toh-e, A. (1990b) IRA2, a second gene of *Saccharomyces cerevisiae* that encodes a protein with a domain homologous to mammalian ras GTPase-activating protein. *Mol Cell Biol*, **10**, 4303-4313.
- Taplick, J., Kurtev, V., Lagger, G. and Seiser, C. (1998) Histone H4 acetylation during interleukin-2 stimulation of mouse T cells. *FEBS Lett*, **436**, 349-352.

- Taverna, S.D., Li, H., Ruthenburg, A.J., Allis, C.D. and Patel, D.J. (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol*, **14**, 1025-1040.
- Thaminy, S., Newcomb, B., Kim, J., Gatbonton, T., Foss, E., Simon, J. and Bedalov, A. (2007) Hst3 is regulated by Mec1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. *J Biol Chem*, **282**, 37805-37814.
- Thomas, J.O. and Kornberg, R.D. (1975a) Cleavable cross-links in the analysis of histone-histone associations. *FEBS Lett*, **58**, 353-358.
- Thomas, J.O. and Kornberg, R.D. (1975b) An octamer of histones in chromatin and free in solution. *Proc Natl Acad Sci U S A*, **72**, 2626-2630.
- Toda, T., Cameron, S., Sass, P. and Wigler, M. (1988) SCH9, a gene of *Saccharomyces cerevisiae* that encodes a protein distinct from, but functionally and structurally related to, cAMP-dependent protein kinase catalytic subunits. *Genes Dev*, **2**, 517-527.
- Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K. and Wigler, M. (1985) In yeast, RAS proteins are controlling elements of adenylate cyclase. *Cell*, **40**, 27-36.
- Tse, C., Georgieva, E.I., Ruiz-Garcia, A.B., Sendra, R. and Hansen, J.C. (1998) Gcn5p, a transcription-related histone acetyltransferase, acetylates nucleosomes and folded nucleosomal arrays in the absence of other protein subunits. *J Biol Chem*, **273**, 32388-32392.
- van Attikum, H. and Gasser, S.M. (2005) The histone code at DNA breaks: a guide to repair? *Nat Rev Mol Cell Biol*, **6**, 757-765.
- Vignali, M., Steger, D.J., Neely, K.E. and Workman, J.L. (2000) Distribution of acetylated histones resulting from Gal4-VP16 recruitment of SAGA and NuA4 complexes. *Embo J*, **19**, 2629-2640.
- Vogelauer, M., Rubbi, L., Lucas, I., Brewer, B.J. and Grunstein, M. (2002) Histone acetylation regulates the time of replication origin firing. *Mol Cell*, **10**, 1223-1233.
- Vogelauer, M., Wu, J., Suka, N. and Grunstein, M. (2000) Global histone acetylation and deacetylation in yeast. *Nature*, **408**, 495-498.
- Walter, W., Clynes, D., Tang, Y., Marmorstein, R., Mellor, J. and Berger, S.L. (2008) 14-3-3 interaction with histone H3 involves a dual modification pattern of phosphoacetylation. *Mol Cell Biol*, **28**, 2840-2849.
- Wang, Y., Pierce, M., Schneper, L., Guldal, C.G., Zhang, X., Tavazoie, S. and Broach, J.R. (2004) Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. *PLoS Biol*, **2**, E128.
- Waterborg, J.H. (2000) Steady-state levels of histone acetylation in *Saccharomyces cerevisiae*. *J Biol Chem*, **275**, 13007-13011.
- Waterborg, J.H. (2001) Dynamics of histone acetylation in *Saccharomyces cerevisiae*. *Biochemistry*, **40**, 2599-2605.

- White, C.L., Suto, R.K. and Luger, K. (2001) Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions. *Embo J*, **20**, 5207-5218.
- Wilson, J.M., Le, V.Q., Zimmerman, C., Marmorstein, R. and Pillus, L. (2006) Nuclear export modulates the cytoplasmic Sir2 homologue Hst2. *EMBO Rep*, **7**, 1247-1251.
- Winter, S., Simboeck, E., Fischle, W., Zupkovitz, G., Dohnal, I., Mechtler, K., Ammerer, G. and Seiser, C. (2008) 14-3-3 proteins recognize a histone code at histone H3 and are required for transcriptional activation. *Embo J*, **27**, 88-99.
- Wood, A., Schneider, J., Dover, J., Johnston, M. and Shilatifard, A. (2003) The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *J Biol Chem*, **278**, 34739-34742.
- Wyce, A., Xiao, T., Whelan, K.A., Kosman, C., Walter, W., Eick, D., Hughes, T.R., Krogan, N.J., Strahl, B.D. and Berger, S.L. (2007) H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. *Mol Cell*, **27**, 275-288.
- Wysocka, J., Swigut, T., Xiao, H., Milne, T.A., Kwon, S.Y., Landry, J., Kauer, M., Tackett, A.J., Chait, B.T., Badenhorst, P., Wu, C. and Allis, C.D. (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature*, **442**, 86-90.
- Xiao, T., Hall, H., Kizer, K.O., Shibata, Y., Hall, M.C., Borchers, C.H. and Strahl, B.D. (2003) Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. *Genes Dev*, **17**, 654-663.
- Xiao, T., Kao, C.F., Krogan, N.J., Sun, Z.W., Greenblatt, J.F., Osley, M.A. and Strahl, B.D. (2005) Histone H2B ubiquitylation is associated with elongating RNA polymerase II. *Mol Cell Biol*, **25**, 637-651.
- Xue, Y., Batlle, M. and Hirsch, J.P. (1998) GPR1 encodes a putative G protein-coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway. *Embo J*, **17**, 1996-2007.
- Ye, J., Ai, X., Eugeni, E.E., Zhang, L., Carpenter, L.R., Jelinek, M.A., Freitas, M.A. and Parthun, M.R. (2005) Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly. *Mol Cell*, **18**, 123-130.
- Zaman, S., Lippman, S.I., Schneper, L., Slonim, N. and Broach, J.R. (2009) Glucose regulates transcription in yeast through a network of signaling pathways. *Mol Syst Biol*, **5**, 245.
- Zhou, J., Chau, C., Deng, Z., Stedman, W. and Lieberman, P.M. (2005) Epigenetic control of replication origins. *Cell Cycle*, **4**, 889-892.
- Zhou, X., Richon, V.M., Rifkind, R.A. and Marks, P.A. (2000) Identification of a transcriptional repressor related to the noncatalytic domain of histone deacetylases 4 and 5. *Proc Natl Acad Sci U S A*, **97**, 1056-1061.

Chapter 2: Materials and Methods

Material in this chapter has been published:

Friis, R.M., Wu, B.P., Reinke, S.N., Hockman, D.J., Sykes, B.D. and Schultz, M.C. (2009b) A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. *Nucleic Acids Res*, **37**, 3969-3980.

2.1 Yeast strains

Table 2.1 lists the strains used in this study. We performed gene deletion by one-step disruption using a PCR fragment amplified from plasmid pFA6a-kanMX6 of (Longtine et al., 1998) with primers appropriate for the targeted gene. Strains we generated were confirmed by PCR using multiple primer sets, including one designed to amplify an internal region of the targeted ORF. When necessary, PCR results were further validated by diagnostic restriction digestion of PCR products. Respiratory competence of all strains was confirmed by their ability to grow on YP 3% glycerol. All p^0 strains (strains lacking mitochondrial DNA (mtDNA)) were created by ethidium bromide treatment of appropriate p^+ strains (strains with mtDNA) as described by Fox *et al.* (1991). Aliquots of cells from SP cultures of temperature sensitive mutants were tested for temperature sensitivity at the conclusion of an experiment in order to exclude results obtained from populations in which enrichment for presumed spontaneous revertants was occurring.

2.2 Yeast culture

Cells were cultured in yeast extract-bactopeptone-2% dextrose (Treco and Lundblad, 1993) at room temperature (22-26°C). Fresh overnight cultures were used to inoculate YPD to 1×10^6 cells/mL. Cells were grown for eight days before nutrient addition. In contrast to previous nutrient-response studies in which spent medium was replaced with new nutrients in water (Dong and Xu, 2004; Granot and Snyder, 1993), we typically added solid nutrients directly to SP cultures. This strategy was adopted in order to limit the environmental change due to nutrient addition. The 'amino acid' addition was: 0.05 g $(\text{NH}_4)_2\text{SO}_4$, 0.017 g Yeast Nitrogen Base without amino acids and ammonium sulfate, and 0.013 g complete dropout powder per 10 mL culture. This mixture provided the indicated ingredients at the concentration used to freshly prepare complete minimal medium (Treco and Lundblad, 1993).

Because the viability of cells in SP cultures declines rapidly if they are incubated at low concentration in 2% glucose in water (Granot and Snyder, 1993), it is possible that glucose induction of histone acetylation is a consequence of physiological misregulation that confers poor plating efficiency. Two

experiments argue against this possibility. First, while G0 and non-quiescent cells isolated from SP cultures differ in their colony forming capacity (~ 100 and 36% respectively at 7 d in culture; (Allen et al., 2006)), both support robust H3/H4 acetylation when fed glucose (Figure 3.4 A). Second, under the conditions we use, viability at 24 h is essentially identical in control and glucose-fed SP cells (Figure 3.1B). Note that cell viability drops at later time points after glucose refeeding (Figure 3.1B). This finding is consistent with previous evidence that glucose triggers physiological changes that are lethal in the absence of the full spectrum of nutrients required for proliferation (Herman, 2002). The precise nature of these changes remains unknown.

Drugs were added to cultures 30 minutes before nutrient refeeding. In experiments involving temperature sensitive strains the temperature up-shift protocol started with pelleting of the cells from 40% of the original culture. The supernatant was split in two and either heated to 65°C or left at room temperature (25°C). The cells in the pellet were immediately recombined with the reserved 60% of the culture (the reserved cells); adding back spent medium to the recombined 'pelleted +

reserved' aliquot restored the original OD₆₀₀ for refeeding. While spent medium was allowed to equilibrate to 25 or 65°C, the recombined aliquots were split into four treatment flasks each (room temperature or 40°C, untreated or treated). A volume of equilibrated spent medium (65°C for 40°C flasks or 25°C for room temperature) equal to 53% of the volume of the recombined aliquot was added to each such aliquot and the resultant treatment cultures transferred to water-bath shakers set to 25°C or 40°C. For example, addition of 5.3 mL of 65°C spent media to 10 mL of 25°C culture allowed virtually instant shift to 39.6°C. Treatment cultures were incubated for at least 30 minutes at room temperature or 40°C before initiation of glucose refeeding. Cells were harvested at the designated time points following nutrient addition. All experiments were performed at least three times, sometimes with minor variations between replicates that did not affect any of our interpretations.

2.3 Fractionation of SP cultures

Equilibrium gradient centrifugation of SP cells was performed at 22°C according to (Allen et al., 2006), with the following minor modifications. Five mL aliquots of cells were sonicated for 5 seconds before pelleting and resuspension at 10⁹

cells/0.5 mL in 50 mM Tris-HCl adjusted to pH 4 with filter-sterilized 0.5 M succinic acid (note that during an 8 d culture the medium drops from pH ~6 to pH ~4). 10 mL gradients of Percoll PLUS adjusted to 167 mM NaCl and pH 4 were overlain with 10^9 cells in 15 mL Corex tubes. Cell fractions were collected from the top of the gradient and counted in a hemacytometer after sonication of an appropriately diluted aliquot. Cell fractions were then diluted with 5 vol 50 mM Tris-HCl (pH 4), pelleted in 15 mL Corex tubes (Beckman Avanti 30 centrifuge with F0650 rotor, 2 m at $19,000 \times g$), and resuspended in spent YPD (collected from the starting 8d culture) at the concentration cells had obtained in the 8d culture, or at 5×10^8 cells/mL (the typical cell concentration of 8 d cultures). Cells were acclimated in YPD for 30 min at room temperature on a rotator before harvesting (control samples) or addition of glucose to 2% vol/vol from a 40% stock in water.

2.4 Metabolic labeling

The labeling protocol developed for SP cells by (Fuge et al., 1994) was modified as follows. Each sample for labeling included 2.5×10^7 G0 cells (fractionation as above) that had been resuspended in spent YPD to the density of the 8 d culture

from which they were isolated. All treatments were performed at room temperature. Cycloheximide or the drug vehicle was added to cells 30 min prior to addition of glucose (2% vol/vol final concentration, from a 40% aqueous stock) or water that also contained 47.6 μCi of [^{35}S]methionine/[^{35}S]cysteine aqueous labeling mix (NEG-772 EasyTagTM Express, PerkinElmer). Isolated proteins were resolved in 15% SDS-PAGE gels, which were then impregnated with EN³HANCE (NEN) and washed in 3% glycerol/20% methanol prior to drying and exposure to film.

2.5 Drug treatment

Drugs were added to cultures in a constant final volume of the drug vehicle, and control cultures received the same volume of vehicle alone. Rapamycin (kind gift of Wyeth-Ayerst) was added from a 2 mM stock dissolved in 10% Tween 20, 90% ethanol. C3-1'-naphthyl-methyl (1NM-PP1) (Calbiochem Cat. No. 529581) was added to 0.1 μM from a 3 mM stock dissolved in dimethyl sulfoxide (DMSO). Cycloheximide (Sigma C-7698) was added to 100 $\mu\text{g/ml}$ from a 10 mg/mL stock freshly prepared in ethanol. This dosage has been employed by others to study

the phenotypic effects of blocking global protein synthesis in yeast (for example (Mitchell and Tollervey, 2003)) and was considered appropriate in view of evidence that SP cells are much more resistant to the overt toxic effects of cycloheximide than are log phase cells (Paz and Choder, 2001). Addition of cycloheximide did not affect the cell density or the flow cytometry profiles of cultures which also received glucose (data not shown).

2.6 Cell analysis and determination of glucose concentration of the culture medium

Sonicated cells were counted using a Coulter Z2 Particle Count and Size Analyzer according to the manufacturer's instructions. Flow cytometry was performed with a FACScan instrument (Becton Dickinson) as described (Stuart and Wittenberg, 1998). Cell viability was assayed by plating appropriate dilutions of cells on YPD in triplicate, and counting colonies after 4 days growth at room temperature. Glucose measurements were made using an Ascencia EliteTMXL Blood Glucose Meter (Bayer). A standard curve was produced by dilution of glucose into fresh YP medium. The medium from yeast cultures was clarified by centrifugation (16,000 x g, 30

seconds) and a dilution series was prepared in YP for application of approximately 20 μ L aliquots (volume of a drop of human blood) to test strips. This assay is linear from 1.1 to 13.9 mmol glucose/L in the YP-diluted sample applied to the test strip; using our assay glucose concentration cannot be reliably measured outside this range.

2.7 Fractionation of soluble histones from histones in chromatin

The method of (Gunjan and Verreault, 2003) was used. Briefly, cells were spheroplasted with 0.5 mg/mL Zymolyase 100T for 15 minutes at 30°C and lysed with Triton X-100. The lysate was centrifuged (21,000 x g for 30 minutes at 4°C); the resultant supernatant and pellet contain the soluble and DNA-associated histones respectively. Proteins in the supernatant were obtained by TCA precipitation and resuspended in SDS-PAGE sample buffer. The pellet was directly resuspended in SDS-PAGE sample buffer.

2.8 Protein isolation and immunoblotting

Protein from 0.5 mL of SP cells was precipitated with 25% trichloroacetic acid after cell lysis in 0.5% β -mercaptoethanol and 0.3 M NaOH (Ramaswamy et al., 2003). The protein was then resuspended in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer at a constant (cell number)/(load buffer volume) ratio. After incubation at 65°C for 20 minutes and vigorous mixing, protein samples in the same volume of load buffer were resolved by SDS-PAGE (15% for histone blots, 6% for Ume6-TAP and Msn2-TAP, 8% for Mig1-TAP, and 10% for Ras2) and electroblotted to nitrocellulose membranes. The membranes were then incubated with primary antibody (in Tris-buffered saline with 4% bovine serum albumin and Tween 20), followed by detection with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse (1:5,000) secondary antibody and enhanced chemiluminescence (Amersham Biosciences, GE Healthcare). Primary antibody dilutions were as follows: bulk H4 antiserum (H196), 1:5,000; bulk H3 antiserum (H193), 1:5,000; H3 K9ac (06-942), 1:1,000; H3 K14ac (07-353), 1:5,000; H3 K18ac (ab1191), 1:1,000; H3 K27ac (07-360), 1:5,000;

K5/K8/K12/K16-acetylated H4 (06-598), 1:1,000; site specific K5-, K8-, K12-, and K16-acetylated H4 (AHP414-AHP417), 1:10,000; K9/K14 acetylated H3 antiserum (06-599), 1:1,000; CBP epitope IgG (07-482), 1:5,000; actin monoclonal (MAB1501), 1:5,000; acetyl-lysine (9441S), 1:1,000; Phospho-AMPK α (Thr172) Cell Signaling #2531, 1:1000; Snf1 (γ K-16) sc-15622, 1:200; Ras2 (γ C-19) sc-6759, 1:200. For quantitation, optical scans of appropriately exposed films were analyzed using NIH Image v 1.61.

2.9 mRNA expression: quantitative real-time reverse transcriptase PCR

Total RNA was isolated from 2×10^9 cells according to (Schmitt et al., 1990), except that the 65°C incubation was extended to 10 min (details at <http://www.biochem.ualberta.ca/SchultzLab>). RNA was resuspended in 50 μ L DEPC-treated water and further purified using an RNeasy mini spin column (Qiagen). The resultant material was precipitated, resuspended in 25 μ L DEPC-treated water, and digested with DNase I (TURBO DNA-free Kit, Ambion) at 10 μ g RNA/50 μ L reaction. This treatment was followed by enzyme inactivation using the DNase Inactivation Reagent

(Ambion). PCR amplification (30 cycles) of 0.5 μ g of each DNase-treated preparation, using reverse transcriptase primers which amplified sequences towards the 3' end of selected genes (below), confirmed DNA removal (PCR reactions were analyzed by 1% agarose gel electrophoresis and ethidium bromide staining). RNA from 2×10^8 cells was used in 50 μ L reverse transcriptase reactions with 600 U SuperScript II (Invitrogen) and 1 μ g random primers. Reactions were performed using the same cell equivalents of RNA to minimize bias due to possible glucose regulation of total RNA content per cell. Note however that expression of *SNR14* RNA, whether normalized to cell equivalents or μ g amounts of RNA, was not affected by glucose refeeding (Vesna C. Nguyen and MCS, data not shown). This finding suggests that glucose regulation of total RNA content per cell is not a significant effect under the conditions studied.

Triplicate real-time reverse transcriptase reactions were performed in the linear range using a Bio-Rad iCycler. Real time PCR reaction mixtures prepared in 25 μ L using the SYBR Green PCR Core Reagents kit (PE Biosystems) contained 1x SYBR Green buffer, 1.5 mM $MgCl_2$, 1 mM dNTP mix with dUTP substituted for dTTP, 1.25 U Taq polymerase (AmpliTaq Gold), 0.2 μ M of each primer, and 2 μ L cDNA. Twenty five μ L reaction mixes (3 for each

cDNA analyzed) were transferred to a 96-well optical plate (iCycler iQ PCR plates, Bio-Rad). Wells were sealed using optical grade tape (iCycler iQ, Bio-Rad), and the plate centrifuged briefly. The thermal cycle protocol used for amplification in the Bio-Rad iCycler was as follows: 95°C (10 minutes), and 40 x 95°C (20 seconds), 56°C (30 seconds), followed by 72°C (30 seconds). Sample fluorescence was measured each cycle during either the 56°C or 72°C step. Melt curve data were then collected immediately, using the thermal protocol 95°C (1 minute), 55°C (1 minute), and then fluorescence was measured as sample temperature was increased by 0.5°C increments every 12 seconds. A mean threshold cycle (TC) was calculated for each sample according to the manufacturer's instructions. For an individual sample, no TC measurement differed from the mean TC by more than 1.7% of the latter. The fold change in expression of an individual mRNA was calculated from the difference between the TC value at 0 and 20 minutes after treatment. Reverse transcriptase primers: *CDC21* Fwd 5'-TAGGCCAGATAGAACAGGCA, *CDC21* Rev 5'-TCGTCATCGCACGTCTTGTAT (324 bp amplicon); *SAM1* Fwd 5'-TGGCCGGTACATTTTATTC, *SAM1* Rev 5'-AATCCAACCTGTGCCTTGGTA (195 bp amplicon). Each primer pair

yielded one DNA fragment of the expected size and no detectable primer dimers.

2.10 mRNA expression: Northern blotting

Expression of *SUT1*/YGL162W mRNA was measured in total RNA isolated from SP control cells (8 d culture), and SP cells harvested at 20 min after 2% glucose refeeding. RNA was isolated as described above and analyzed without spin column purification. ³²P-labeled *SUT1* probe was prepared by random primed labeling of a fragment encompassing the entire gene, obtained by PCR of yeast genomic DNA using primers 5'-GTCCACAAGCATTACAGTAAG (*SUT1* 5' FOR) and 5'-TTCATTAGAGATGCCTTTGTC (*SUT1* 3' REV). Northern blotting of 10 µg aliquots of total RNA was performed according to (Ramaswamy et al., 2003). For quantitation, radioactive band intensity was determined by phosphorimager analysis (Molecular Dynamics Storm 640), using ImageQuant TM to process the data. The phosphorimager results were normalized to the recovery of 18S rRNA, which was detected by ethidium bromide staining of gels, and quantitated by image analysis using the

ChemImager™ Ready system (Alpha Innotech Corp.) and AlphaImager 2200 v. 5.5 software.

2.11 mRNA Expression: microarray analysis

Six hundred mL SDC cultures of wild type BY4741 ρ^+ and ρ^0 cells were grown to 1 OD₆₀₀ at room temperature. Half of each culture was harvested for isolation of total RNA. The remaining half was collected by centrifugation, washed once in the same media lacking glucose (S_C), then resuspended at 1 OD₆₀₀ in S_C and incubated with constant shaking for 4 hours. After 4 hours the remaining culture was harvested for preparation of total RNA. Small samples were taken for western analysis of histone H4 acetylation. Each experiment was performed in triplicate. Figure 5.1 shows the western analysis of one of the three experiments. Total RNA was harvested from an equal number of cells using hot phenol extraction (Schmitt et al., 1990). Total RNA was quantified by A260/280 measurements. Poly (A)⁺ mRNA was isolated from total RNA using a Qiagen Oligotex mRNA kit, and used for the synthesis of double stranded cDNA. Double-stranded cDNA was used to synthesize biotin-labeled cRNA as described in Affymetrix procedures (GeneChip Expression Analysis Technical Manual). The biotin-labeled cRNA

was fragmented to maximize hybridization efficiency and assay sensitivity. At all steps RNA and DNA concentration and purity were evaluated by absorbance at 280nm and 260nm and the A260/A280 ratio. Sample cleanup was performed at each step using the GeneChip Sample Cleanup Module. Biotin Labeled cRNA was hybridized to the *S. cerevisiae* GeneChip YG_S98 (Affymetrix) and stained with streptavidin phycoerythrin conjugate as per Affymetrix protocols. Fluorescence intensity measurements were carried out using GeneChip software. Gene Spring version 6.1 software (Agilent Technologies) and tools for analysis of transcriptional data provided by yeastract.com were used for data analysis (Monteiro et al., 2008; Teixeira et al., 2006).

2.12 Profiling of cellular metabolites by ^1H -NMR spectroscopy

Aliquots equivalent to 7 mL of 20 OD₆₀₀ culture were collected at the indicated time points after glucose refeeding. Cells were pelleted at 9,391 x g for 1 minute in a Beckman Avanti 30 centrifuge (F0650 rotor), resuspended in 10 mL room temperature water and immediately pelleted again for 1 minute.

Cell pellets were flash frozen in liquid nitrogen and stored at -80°C until use.

Frozen cell pellets were resuspended in 900 µL of ice-cold water and transferred to 2 mL screw cap microfuge tubes. Samples were adjusted to 5% trichloroacetic acid (TCA). One mL of 0.5 mm glass beads (BioSpec Products, #11079150) was added to each tube. Two 30 second rounds of bead beating were performed in a BioSpec Mini-Beadbeater at 4,200 rpm with 2 minutes on ice between beatings. Supernatants were collected and clarified by centrifugation at 14,000 rpm for 20 minutes at 4°C (Eppendorf centrifuge 5415C). Samples were adjusted to pH 7 with NaOH, flash frozen in liquid nitrogen, and stored at -80°C until they were lyophilized and resuspended in 570 µL D₂O (99.9%. Isotec Inc., Miamisburg, Ohio), along with 30 µL of 5mM DSS-d₆, 2,2-dimethyl-2-sila 3,3,4,4,5,5-hexadeuteropentane sulphonic acid, (Chenomx Inc., Edmonton, Alberta) as a chemical shift indicator and concentration standard for NMR analysis. pH was recorded and samples were spun at 13000 rpm for 3 minutes in a MSE Micro Centaur centrifuge to remove particulate matter. Five hundred µL of the supernatant were transferred to a 5 mm NMR tube. All spectra were acquired

at 30°C, had an acquisition time of 4 seconds, a preacquisition delay of 1 second, a mixing time of 0.1 second, a sweep width of 7200 Hz and 256 transients.

2.13 Enzymatic measurement of cellular acetate

An aliquot equivalent to 9.5 mL of 22.8 OD₆₀₀ culture was pelleted and washed as for ¹H-NMR analysis of metabolites. Cell lysis was accomplished using a modified version of the procedure of (Lin et al., 2001). Pelleted cells were resuspended in 500 µL ice cold water, which was then adjusted to 0.24 M NaOH 1 mM EDTA. Cells were next incubated on ice for 10 min before addition of 20 µL of concentrated HCl. Samples were then incubated at 65°C for 30 minutes to eliminate remaining enzymatic activity, and spun at 14,000 rpm in an Eppendorf centrifuge 5415C at 4°C for 20 minutes. The supernatant was transferred to fresh tubes and neutralized with 100 µL of 1 M Tris Base. Samples were spun again at 14,000 rpm for 10 minutes to remove precipitate. Supernatant was collected and acetate was detected using an enzymatic assay kit (Enzytec™ Acetic Acid Kit from Scil Diagnostics GmbH) according to the manufactures' instructions.

Table 2.1. Yeast strains used in this study.

Strain	Back-ground	Relevant genotype	Original publication	Source
BY4741	S288c	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Brachmann et al., 1998 (Brachmann et al., 1998)	Open Biosystems
<i>RPB1</i>	BY4742 (S288c)	<i>MATα his3Δ1 leu2Δ1 lys2Δ0 ura3Δ0</i>	M. Werner-Washburne; MW1531 (BY4742 is from Brachmann et al., 1998) (Brachmann et al., 1998)	M. Werner-Washburne
<i>rpb1-1</i>	S288c	<i>MATa leu2-3,112 lys2 trp1Δ ura3-52 rpb1-1</i>	Nonet et al., 1987 (Nonet et al., 1987)	M. Werner-Washburne
<i>RPD3-TAP</i>	BY4741	BY4741, <i>HIS3::RPD3-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics
<i>rpd3Δ</i>	BY4741	BY4741, <i>rpd3Δ::HIS3MX6</i>	This study	
<i>hat1Δ</i>	BY4741	BY4741, <i>hat1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>sas2Δ</i>	BY4741	BY4741, <i>sas2Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>ESA1-TAP</i>	BY4741	BY4741, <i>HIS3::ESA1-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics
<i>ESA1</i>	S288c	<i>MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52</i>	Clarke et al., 1999 (Clarke et al.,	L. Pillus

			1999); LPY3498	
<i>esa1-414</i>	S288c	<i>MATa esa1Δ::HIS3</i> isolate from LPY2639, with a <i>CEN-esa1-414</i> plasmid (pLP863)	Clarke et al., 1999; LPY3291 (Clarke et al., 1999)	L. Pillus
<i>esa1-L254P</i>	S288c	<i>MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52 esa1Δ::HIS3 esa1-L254P::URA3</i>	Clarke et al., 1999; LPY3500 (Clarke et al., 1999)	L. Pillus
<i>gcn5Δ</i>	BY4741	BY4741, <i>gcn5Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>hpa2Δ</i>	BY4741	BY4741, <i>gcn5Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>sas3Δ</i>	BY4741	BY4741, <i>sas3Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>EPL1</i>	BY4741		Boudreault et al., 2003 (Boudreault et al., 2003)	J. Côté
<i>EPL1-HA</i>	BY4741	<i>epl1Δ::kanMX</i> with pPHEL: <i>HA-EPL1</i> , <i>EPL1</i> promoter, <i>ARS/CEN</i> , <i>LEU2</i>	Boudreault et al., 2003 (Boudreault et al., 2003)	J. Côté
<i>epl1(1-485)-HA</i>	BY4741	<i>epl1Δ::kanMX</i> with pPNEL: <i>HA-EPL1(1-485)</i> , <i>EPL1</i> promoter, <i>ARS/CEN</i> , <i>LEU2</i>	Boudreault et al., 2003 (Boudreault et al., 2003)	J. Côté
<i>spt7Δ</i>	BY4741	BY4741, <i>spt7Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>ahc1Δ</i>	BY4741	BY4741, <i>ahc1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>spt8Δ</i>	BY4741	BY4741, <i>spt8Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et	Open Biosystems

			al., 1999)	
<i>rtg2Δ</i>	BY4741	BY4741, <i>rtgΔ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>TRA1</i>		<i>MATa tra1Δ::LEU2 [pTRA1 URA CEN]</i>	Brown et al., 2001 (Brown et al., 2001); YCB650	J. Workman
<i>tra1-2</i>		YCB650, <i>tra1-ts#2B [pCB155 (tra1-2 TRP CEN)]</i>	Brown et al., 2001; YCB655 (Brown et al., 2001)	J. Workman
<i>chd1Δ</i>	BY4741	BY4741, <i>chd1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>tra1-2 chd1Δ</i>		<i>tra1-2 chd1Δ::kanMX6</i> (from YCB655)	This study	
<i>asf1Δ</i>	BY4741	BY4741, <i>asf1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>ACS1-TAP</i>	BY4741	BY4741, <i>HIS3::ACS1-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics
<i>ACS2-TAP</i>	BY4741	BY4741, <i>HIS3::ACS2-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics
<i>acs1Δ</i>	BY4741	BY4741, <i>acs1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	J. Boeke
<i>acs2-Ts1</i>	BY4741	BY4741, <i>acs2Δ0::HygMX [pHT215, acs2Ts1 CEN URA3]</i>	Takahashi et al., 2006 (Takahashi et al., 2006); YTH652	J. Boeke
<i>acs1Δ</i>	BY4741	<i>acs2-Ts1, acs1-Δ0::HIS3</i>	Takahashi et al.,	J. Boeke

<i>acs2-Ts1</i>			2006 (Takahashi et al., 2006); YTH729	
A364A	A364A	<i>MATa gal1 his7 try1 lys2 ade1 ura1 ade2</i>	A364A	ATCC # 204626
<i>cdc19-1</i>	A364A	<i>MATa ade1 ade2 ura1 his7 lys2 try1 gal1 cdc19-1 (pyk1) ade</i>	395	ATCC # 208580
<i>fpr1Δ</i>	BY4741	BY4741, <i>fpr1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>hmo1Δ</i>	BY4741	BY4741, <i>hmo1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>cdc25-1</i>	A364A	<i>MATa ade1 ade2 ura1 his7 lys2 try1 gal1 cdc25-1</i>	321	ATCC# 208586
<i>gpr1Δ</i>	BY4741	BY4741, <i>gpr1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>gpa2Δ</i>	BY4741	BY4741, <i>gpa2Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>cdc35-1</i>	A364A	<i>MATa ade1 his7 trp1 ura1 arg (not arg4) cdc35-1</i>	BR214-4a	ATCC# 208600
<i>snf1Δ</i>	BY4741	BY4741, <i>snf1Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems
<i>MIG1-TAP</i>	BY4741	BY4741, <i>HIS3::MIG1-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics

<i>MSN2-TAP</i>	BY4741	BY4741, <i>HIS3::MSN2-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics
<i>UME6-TAP</i>	BY4741	BY4741, <i>HIS3::UME6-TAP</i>	Ghaemmaghami et al., 2003 (Ghaemmaghami et al., 2003)	Research Genetics
<i>ras2Δ</i>	BY4741	BY4741, <i>ras2Δ::kanMX6</i>	Winzeler et al., 1999 (Winzeler et al., 1999)	Open Biosystems

2.14 References

- Allen, C., Buttner, S., Aragon, A.D., Thomas, J.A., Meirelles, O., Jaetao, J.E., Benn, D., Ruby, S.W., Veenhuis, M., Madeo, F. and Werner-Washburne, M. (2006) Isolation of quiescent and nonquiescent cells from yeast stationary-phase cultures. *J Cell Biol*, **174**, 89-100.
- Boudreault, A.A., Cronier, D., Selleck, W., Lacoste, N., Utley, R.T., Allard, S., Savard, J., Lane, W.S., Tan, S. and Cote, J. (2003) Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev*, **17**, 1415-1428.
- Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*, **14**, 115-132.
- Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S. and Workman, J.L. (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science*, **292**, 2333-2337.
- Clarke, A.S., Lowell, J.E., Jacobson, S.J. and Pillus, L. (1999) Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol Cell Biol*, **19**, 2515-2526.
- Dong, L. and Xu, C.W. (2004) Carbohydrates induce mono-ubiquitination of H2B in yeast. *J Biol Chem*, **279**, 1577-1580.
- Fox, T.D., Folley, L.S., Mulero, J.J., McMullin, T.W., Thorsness, P.E., Hedin, L.O. and Costanzo, M.C. (1991) Analysis and manipulation of yeast mitochondrial genes. *Methods Enzymol*, **194**, 149-165.
- Fuge, E.K., Braun, E.L. and Werner-Washburne, M. (1994) Protein synthesis in long-term stationary-phase cultures of *Saccharomyces cerevisiae*. *J Bacteriol*, **176**, 5802-5813.
- Ghaemmighami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K. and Weissman, J.S. (2003) Global analysis of protein expression in yeast. *Nature*, **425**, 737-741.
- Granot, D. and Snyder, M. (1993) Carbon source induces growth of stationary phase yeast cells, independent of carbon source metabolism. *Yeast*, **9**, 465-479.
- Gunjan, A. and Verreault, A. (2003) A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in *S. cerevisiae*. *Cell*, **115**, 537-549.
- Herman, P.K. (2002) Stationary phase in yeast. *Curr Opin Microbiol*, **5**, 602-607.
- Lin, S.S., Manchester, J.K. and Gordon, J.I. (2001) Enhanced gluconeogenesis and increased energy storage as hallmarks of aging in *Saccharomyces cerevisiae*. *J Biol Chem*, **276**, 36000-36007.
- Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachet, A., Philippsen, P. and Pringle, J.R. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast*, **14**, 953-961.
- Mitchell, P. and Tollervey, D. (2003) An NMD pathway in yeast involving accelerated deadenylation and exosome-mediated 3'→5' degradation. *Mol Cell*, **11**, 1405-1413.

- Monteiro, P.T., Mendes, N.D., Teixeira, M.C., d'Orey, S., Tenreiro, S., Mira, N.P., Pais, H., Francisco, A.P., Carvalho, A.M., Lourenco, A.B., Sa-Correia, I., Oliveira, A.L. and Freitas, A.T. (2008) YEASTRACT-DISCOVERER: new tools to improve the analysis of transcriptional regulatory associations in *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **36**, D132-136.
- Nonet, M., Scafe, C., Sexton, J. and Young, R. (1987) Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. *Mol Cell Biol*, **7**, 1602-1611.
- Paz, I. and Choder, M. (2001) Eukaryotic translation initiation factor 4E-dependent translation is not essential for survival of starved yeast cells. *J Bacteriol*, **183**, 4477-4483.
- Ramaswamy, V., Williams, J.S., Robinson, K.M., Sopko, R.L. and Schultz, M.C. (2003) Global control of histone modification by the anaphase-promoting complex. *Mol Cell Biol*, **23**, 9136-9149.
- Schmitt, M.E., Brown, T.A. and Trumpower, B.L. (1990) A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **18**, 3091-3092.
- Stuart, D. and Wittenberg, C. (1998) CLB5 and CLB6 are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. *Genes Dev*, **12**, 2698-2710.
- Takahashi, H., McCaffery, J.M., Irizarry, R.A. and Boeke, J.D. (2006) Nucleocytoplasmic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription. *Mol Cell*, **23**, 207-217.
- Teixeira, M.C., Monteiro, P., Jain, P., Tenreiro, S., Fernandes, A.R., Mira, N.P., Alenquer, M., Freitas, A.T., Oliveira, A.L. and Sa-Correia, I. (2006) The YEASTRACT database: a tool for the analysis of transcription regulatory associations in *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **34**, D446-451.
- Treco, D.A. and Lundblad, V. (1993) Basic techniques of yeast genetics. In Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (eds.), *Current Protocols in Molecular Biology*. John Wiley and Sons, New York, Vol. 2, pp. 13.11.11-13.11.17.
- Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., Chu, A.M., Connelly, C., Davis, K., Dietrich, F., Dow, S.W., El Bakkoury, M., Foury, F., Friend, S.H., Gentlen, E., Giaever, G., Hegemann, J.H., Jones, T., Laub, M., Liao, H., Davis, R.W. and et al. (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science*, **285**, 901-906.

Chapter3: Glucose regulation of overall histone acetylation

Material in this chapter has been published:

Friis, R.M., Wu, B.P., Reinke, S.N., Hockman, D.J., Sykes, B.D. and Schultz, M.C. (2009b) A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. *Nucleic Acids Res*, **37**, 3969-3980.

3.1 Introduction

Histones H2A, H2B, H3 and H4, the protein components of the nucleosome core particle, are subject to numerous chemically distinct post-translational modifications. In terms of function and regulation the best characterized amongst these modifications is reversible acetylation of lysine residues in the conserved histone amino-terminal tails. Regulation of histone acetylation is an integral component of many processes which involve DNA including histone deposition and remodeling during replication, the timing of firing of DNA replication origins, DNA damage repair as well as induction and repression of transcription (Aggarwal and Calvi, 2004; Antequera, 2004; Ehrenhofer-Murray, 2004; Kurdistani and Grunstein, 2003; van Attikum and Gasser, 2005).

Our experiments extend previous studies in which it was shown by immunoblotting analysis of total cellular proteins that overall H3/H4 acetylation declines as yeast cells progress into stationary phase (SP) in response to nutrient depletion from their environment (Ramaswamy et al., 2003; Sandmeier et al., 2002). Conversely, SP cells inoculated into fresh medium give rise to an expanding population with a relatively high level of

histone acetylation (data not shown). Since glucose refeeding in SP triggers gross morphological changes characteristic of re-entry into a proliferative state (Granot and Snyder, 1991), we reasoned that it might also induce overall H3/H4 acetylation. Here we show that glucose refeeding indeed triggers robust acetylation of nucleosomal H3 (at K9) and H4 (at K5, 8, 12) in SP cells. Based upon the known cell cycle mediated regulation of histone acetylation, if glucose does trigger cell cycle re-entry, the induction of histone acetylation may result from the acetylation of newly synthesised histones concomitant with entry into S-phase. Transcription of histones peaks in S-phase leading to increases in protein levels of H3 and H4, accompanied by acetylation of H4 by HAT1 and H3 by Gcn5 (Adkins et al., 2007; Moll and Wintersberger, 1976; Sobel et al., 1995). Herein we analyze the possibility that S-phase re-entry could be the driving force for overall increases in histone acetylation seen in refed SP cells.

3.2 Results and Discussion

3.2.1 Glucose regulation of H3/H4 acetylation in SP cells

We explored the nutrient response of SP cells in 8 d cultures as outlined in Figure 3.1 A. At set times after nutrient addition, the glucose content of the medium and cell viability were determined (Figure 3.1 B), and cell aliquots were processed for automated counting, flow cytometry (to monitor DNA content) and isolation of total protein. Effects of refeeding on H3/H4 metabolism were assessed by immunoblotting (details in Chapter2: materials and methods). Briefly, 'acetylated H4' (H4ac) was detected with an antibody specific for H4 acetylated on at least one of K5, 8, 12, or 16, 'acetylated H3' (H3ac) was detected using an antibody raised against a H3 tail peptide acetylated at K9 and K14, and protein loading was monitored using antibodies against bulk H3/H4 or actin. Changes in acetylation revealed by the H3ac/H4ac antibodies were also readily detected by an anti-acetyl lysine antibody.

We observed robust H4 acetylation following simultaneous refeeding with glucose and amino acids. Acetylation peaks at 4

h (Figure 3.2 A, lanes 9, 12) and is maintained for up to 24 h (Figure 3.2 A, and data not shown). After peaking, H4 acetylation slowly declines to below the starting level (Figure 3.2 A, lanes 1, 24). These changes were not accompanied by obvious changes in steady state bulk H4 expression. Parallel feeding revealed: 1) that increased acetylation in cells fed glucose and amino acids is due to the provision of glucose (Figure 3.2 A, lanes 9-12; average glucose induction corrected for bulk H4 recovery = 4.7, n=3), and 2) that the persistence of H4ac is positively correlated with the glucose dose (Figure 3.2 B, compare lanes 11, 12 to 19, 20; glucose measurements are in Figure 3.1 B).

Probing with site-specific antibodies revealed that fluctuations in acetylation detected by the H4ac antibody (Figure 3.2 A, B) are due to acetylation and deacetylation at K5, K8 and K12, but not K16 (Figure 3.2 C). Figure 3.2 C also shows that H3 K9/K14 acetylation is regulated similarly to H4 K5, 8, and 12 acetylation. A more detailed analysis showing glucose induction of H3 K9, K14, K18 and K27 acetylation is presented in Figure 3.2 D. We conclude that glucose triggers transient acetylation at K5, 8 and 12 of H4 and at K9, 14, 18 and 27 of H3 in SP cells.

3.2.2 Glucose induction of H3/H4 acetylation in SP is a replication-independent phenomenon

The pattern of induction and repression of H3/H4 acetylation following glucose refeeding, and the residue-specificity of acetylation, resemble the regulation of overall H3/H4 acetylation during G1 to M cell cycle progression (Adkins et al., 2007). Perhaps then glucose induction of acetylation in SP is coupled to nuclear DNA replication? Several observations argue against this possibility. Cell number does not increase after glucose refeeding (Figure 3.3 A), and during the time when histone acetylation increases in glucose-fed cultures, the region of the cellular DNA content curve that normally develops a shoulder upon S phase entry does not increase in glucose fed cells (Figure 3.3 B, arrows) unless they also receive amino acids (Figure 3.3 B, arrowheads). We conclude that induction of DNA replication is not associated with glucose induction of H3/H4 acetylation in SP. Werner-Washburne and colleagues have shown that SP cultures contain non-cycling 'G0' cells and very slowly dividing 'non-quiescent' cells (Allen et al., 2006). Since G0 cells isolated from 8 d cultures support glucose induction of H4ac, the response does not require ongoing replication (Figure 3.4).

Saccharomyces cerevisiae enter SP from G1 and exit SP into G1 (Gray et al., 2004). Progression through the cell cycle is driven by the cyclin-dependent kinase Cdc28. Different cyclins associate with and activate Cdc28 at specific points in the cell cycle to direct progression past these points. In order to corroborate our flow cytometry data that glucose does not trigger normal progression into S-phase we examine the protein levels of two cyclins which would be expressed during the progression from G1 to S-phase. Cln2 is a G1 cyclin involved in the G1 to S-phase transition and Clb5 is a B-type cyclin involved in initiation of DNA replication. Expression of both of these peak late in G1 (Cross et al., 1994; Schwob and Nasmyth, 1993; Spellman et al., 1998). We used strains harbouring either TAP-tagged Cln2 or Clb5. By 1 hour after glucose refeed when new histone acetylation is readily apparent (Figure 3.2 A lanes 5 and 6) there was no accumulation of Cln2 or Clb5 in glucose fed or unfed cells (Figure 3.4 B lanes 4 and 5). These cells were capable of cell cycle re-entry as an aliquot taken from the original SP cultures gave rise to a population of cycling cells that showed clear accumulation of both Cln2 and Clb5 (Figure 3.4 B lane 6). Collectively these results suggest that glucose induction

of histone acetylation can occur independently of the normal program of DNA replication.

The viability of cells in SP cultures declines rapidly if they are incubated at low concentration in 2% glucose in water. For example, viability declines by about 25% when SP cells are incubated for 24 h in 2% glucose (Granot and Snyder, 1993). This observation raises the possibility that glucose induction of histone acetylation is a consequence of physiological misregulation that conveys poor plating efficiency. Two experiments argue against this possibility. First, while G0 and non-quiescent cells differ in their colony forming capacity (~ 100 and 36% respectively at 7 d in culture; Allen et al., 2006), both support robust H3/H4 acetylation when fed glucose (Figure 3.4 A). Furthermore, under the conditions we use, viability at 24 h is essentially identical in control and glucose-stimulated SP cells (Figure 3.1 B). Therefore induction of acetylation triggered by glucose is not a consequence of entry of cells into a physiological state from which re-proliferation is not possible. Note that viability does drop at later time points after glucose refeeding. This finding is consistent with previous evidence that glucose

triggers physiological changes that are lethal in the absence of the full spectrum of nutrients required for proliferation.

Histone acetylation during S phase is coupled to new histone synthesis (Rocha and Verreault, 2008). Since acetylation triggered by glucose refeeding in SP does not require replication, it is unlikely to be associated with new histone synthesis dependent on G1 to S cell cycle progression cues. Histone expression can be artificially driven to a very high level outside of S phase, and such newly synthesized histones are incorporated into chromatin (Rufiange et al., 2007). Additional evidence exists which showed that transcription of histones could be induced by reinoculating stationary phase cells into fresh media (Drebot et al., 1990). These observations raise the possibility that glucose induction of histone synthesis is important for glucose induction of chromatin acetylation in SP. This prediction was tested by immunoblotting approaches, as used to study new histone synthesis during S phase (Adkins et al., 2007; Berndsen et al., 2008), in combination with metabolic labelling and cycloheximide treatment. Under conditions that repress translation (Figure 3.5 A), we find that cycloheximide does not obviously affect bulk H4 expression at the time of

glucose addition or at 4 h after glucose addition, when acetylation peaks (Figure 3.5 B, lanes 1, 2 and 5, 6). Furthermore, fractionation studies revealed that the free histone pool is extremely small in SP cells, and that glucose strongly induces acetylation of chromatin-associated H4 (Figure 3.5 C). Finally in ^{35}S -methionine/ ^{35}S -cysteine labelling experiments, the band pattern in the gel region where histones migrate reveals no evidence of protein induction (Figure 3.5 D lanes 2, 4). Therefore the results obtained by immunoblotting of total protein extracts likely reflect the regulation of histones that reside in nucleosomes, or become incorporated into nucleosomes. We conclude that histone acetylation in glucose-fed SP cells occurs mostly on pre-existing histones. Our results do not rule out the possibility that acetylation of free histones is a minor mechanism contributing to glucose induction of chromatin acetylation in SP, or that glucose can stimulate acetylation of newly synthesized histones during S phase in actively dividing cells.

Although new histone synthesis is not associated with glucose-induction of acetylation in SP, glucose stimulation of H4 K5, 8, 12 acetylation is dampened when translation is inhibited (Figure 3.5 B). Therefore, while the machinery required for

reacetylation is present in SP, comprehensive glucose induction of acetylation requires new or ongoing synthesis of an unidentified protein factor(s). Since glucose induces transcription in SP cells (Figure 3.6 A) and induction of acetylation is dampened in a RNA polymerase II mutant at the restrictive temperature (Figure 3.6 B), increased synthesis of such factors might also require *de novo* synthesis of their mRNAs.

3.3 Conclusions

The results presented in this chapter clearly demonstrate that SP cells initiate a response upon refeeding that results in overall increases in acetylation at H4 K5, K8 and K12 and H3 K9, K14, K18, and K27 (Figure 3.2 C and D). This response is not dependent upon cell cycle reentry and progression into S-phase. Glucose refeed does not result in cell proliferation, or DNA synthesis (Figure 3.2 A and B). Further, at a time when glucose refeed consistently results in strong induction of overall histone acetylation, two cyclins that are upregulated before cells enter S-phase are not induced (Figure 3.4 B). The effects upon overall histone acetylation appear to be mediated by factors acting upon

nucleosomes rather than free histones. No new histone synthesis is detectable by examination of bulk histone levels by Western blotting, and the soluble (free) pool of histones is vanishingly small in SP and does not seem to be substantially induced during glucose refeed (Figure 3.5 C). Therefore, this system provides a model in which we can examine replication independent control of overall histone acetylation by targeted and untargeted activities.

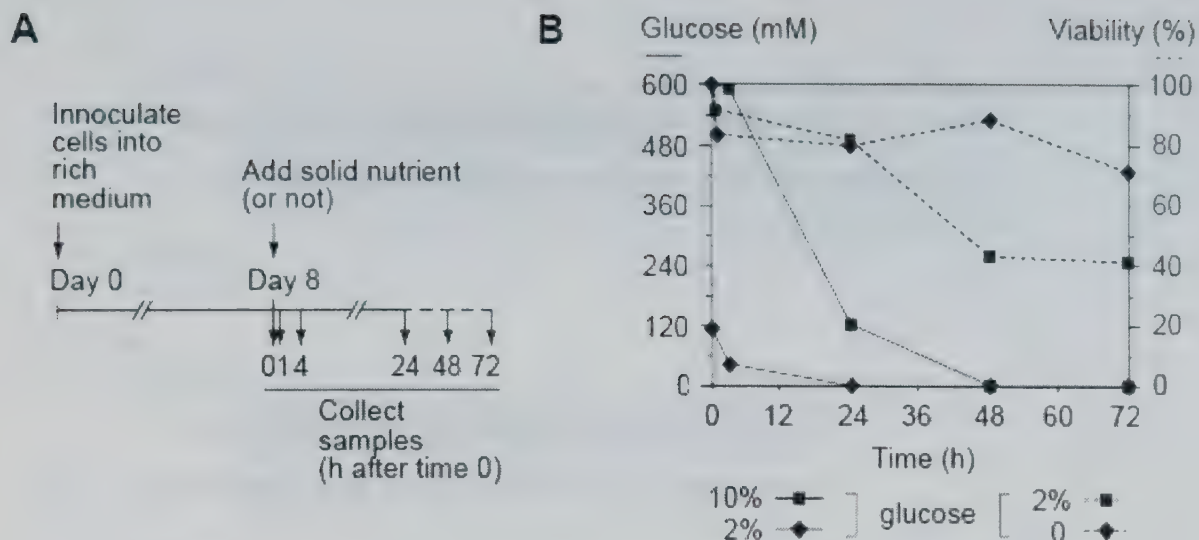


Figure 3.1 Depletion of glucose and cell viability during the course of a refeeding experiment.

(A) Outline of culture/refeeding protocol. Time 0 corresponds to 8 d in culture, before nutrient addition.

(B) Culture medium glucose concentration (solid lines), and cell viability (dashed lines), plotted as a function of time after refeeding. Viability is normalized to 100% live cell recovery at time 0.

The experiment in B was performed by B. Wu partially in consultation with me.

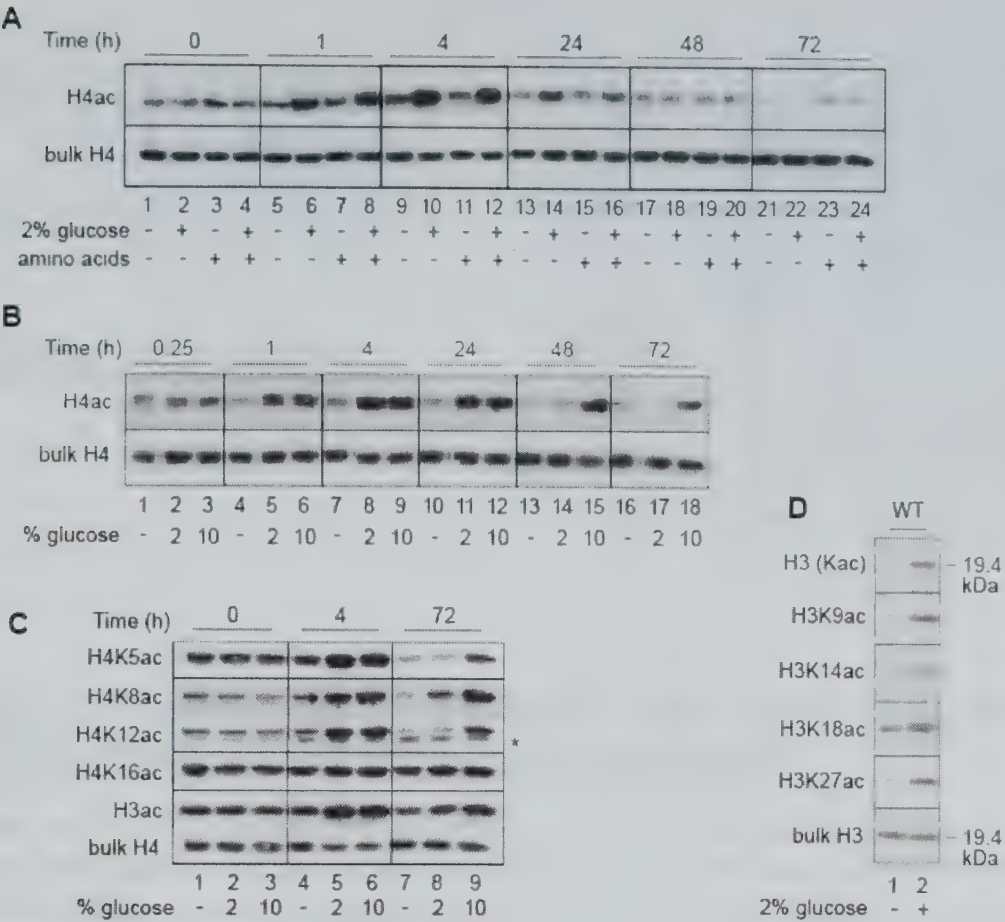


Figure 3.2 Overall histone acetylation on H3 and H4 is stimulated by by glucose not amino acids during nutrient refeed.

(A) Immunoblot analysis of H4 acetylation (H4ac antibody) and bulk H4 expression (loading control) at the indicated time points after nutrient refeeding.

(B) Immunoblot analysis of H4 acetylation and bulk H4 expression at the indicated time points after refeeding with 2 or 10% glucose.

(C) Immunoblot analysis of histone acetylation using residue- and acetylation-specific anti-H4 antibodies, and an antibody raised against an H3 K9ac K14ac tail peptide.

(D) Immunoblot analysis of histone acetylation using residue-and acetylation-specific anti-H3 antibodies.

The experiments in A-C were performed by B. Wu partially in consultation with me.

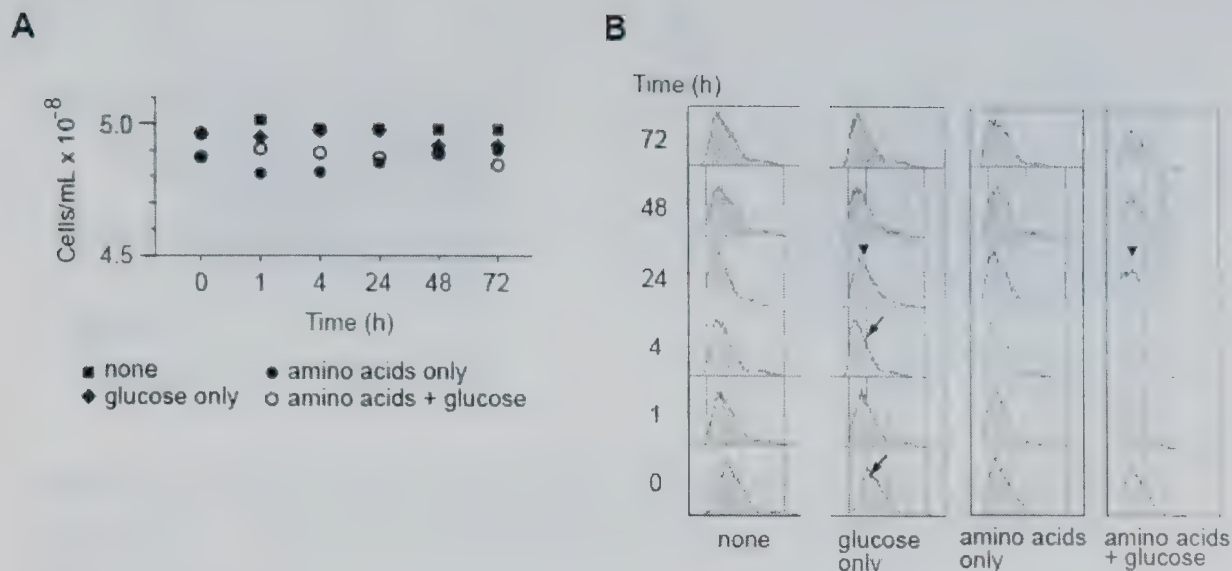


Figure 3.3 Neither cell proliferation nor DNA replication are stimulated by refeeding glucose alone.

(A) Cell density as a function of time after nutrient addition to SP cultures.

(B) Flow cytometry profiles of SP populations at specific time points after nutrient addition. Each profile shows fluorescence intensity on the X axis and cell number on the Y axis; the peak corresponds to 1n DNA content.

These experiments were performed by B. Wu partially in consultation with me.

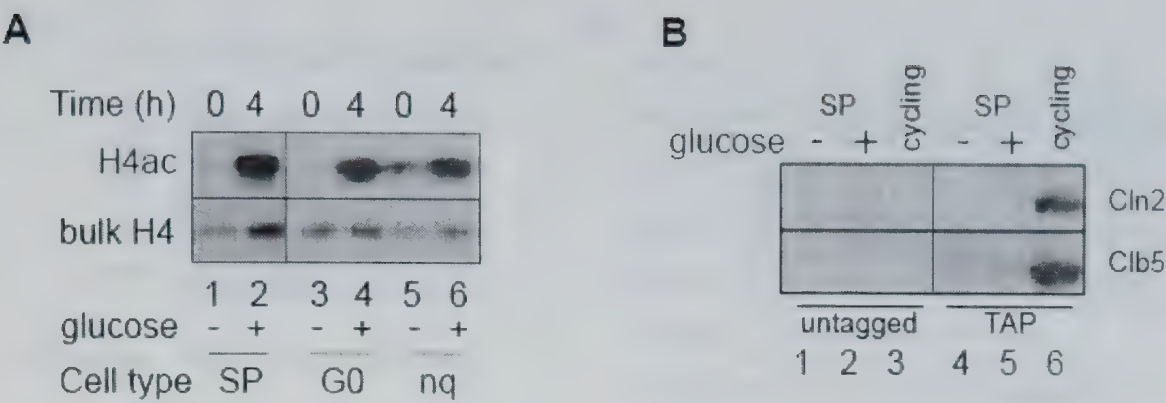


Figure 3.4 Glucose induces acetylation in G0 and non-quiescent cells, and glucose refeeding does not drive new synthesis of Cln2 or Clb5.

(A) Glucose response of G0 and non-quiescent cells isolated from an 8 d culture. Immunoblotting analysis of H4 acetylation in the G0 and non-quiescent (nq) cell populations isolated from a mixed stationary phase culture (SP). Bulk H4 is the loading control. G0 populations, with a relatively low budding index (mean = 4.1%, n = 3), supported glucose induction of H3/H4 acetylation as well as non-quiescent (mean budding index = 31.7%, n =3) and mixed SP populations. Bulk H4 is the loading control.

(B) Anti-CBP Immunoblotting analysis of TAP-tagged Cln2 and Clb5 during glucose refeed. Unfed and glucose fed cells were harvested 1 hour after glucose refeed. An aliquot of the original SP cultures later used for refeed was inoculated into fresh YPD and harvested 6 hours later to obtain a cycling population.

The cell fractions for the experiments in (A) were provided by M. Schultz

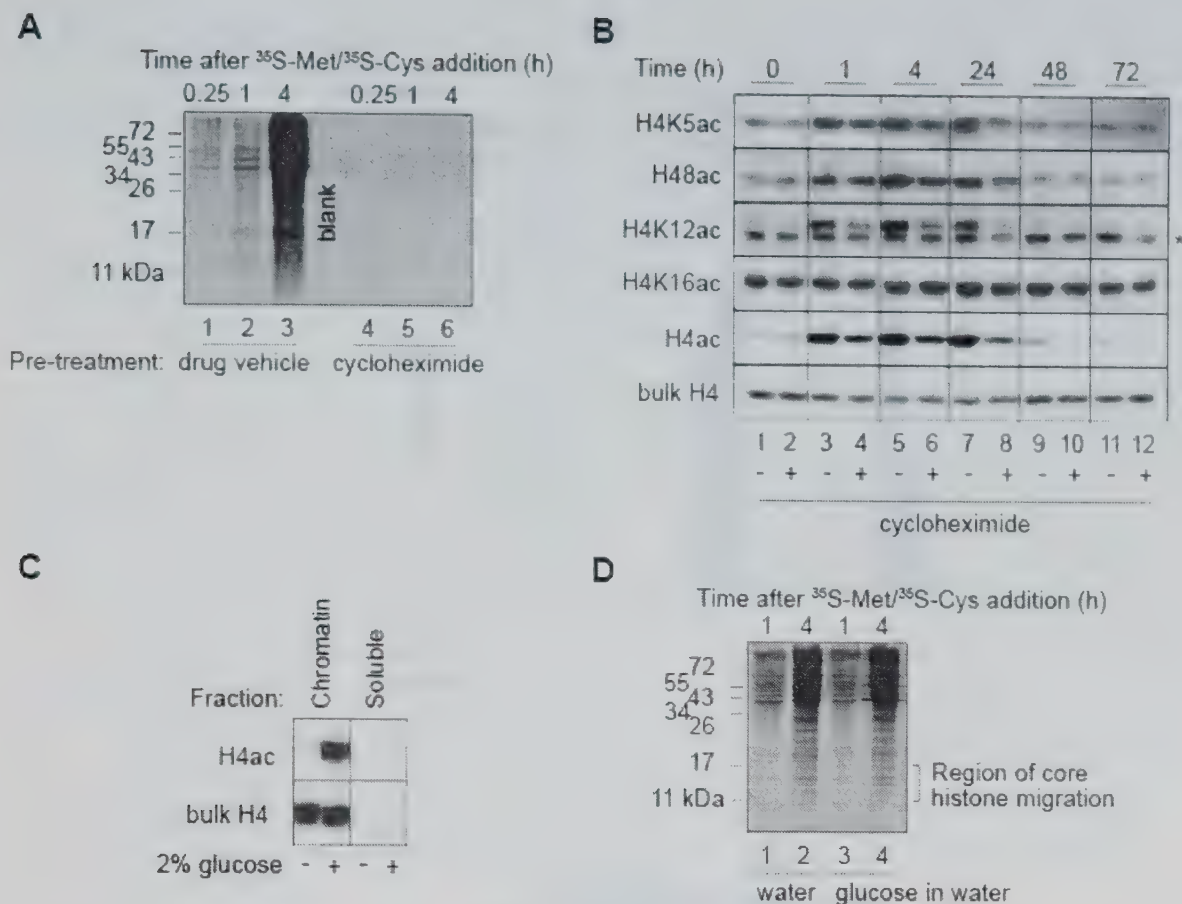


Figure 3.5 Overall acetylation in response to glucose refeed increases on nucleosomal histones even when protein translation is inhibited.

(A) ^{35}S -methionine/ ^{35}S -cysteine incorporation into proteins in G0 cells, in the presence or absence of cycloheximide. Proteins resolved by SDS-PAGE were detected by fluorography.

(B) Immunoblotting analysis of H4 acetylation in glucose-fed cells treated with cycloheximide. H4 acetylation isoforms were detected with the indicated antibodies. The asterisk identifies an unknown band; its intensity is not obviously related to glucose availability.

(C) Immunoblot analysis of acetylated and bulk H4 in the soluble and DNA-associated pools of histones obtained from cells before and after (4 h) glucose refeeding.

(D) Protein synthesis in control and glucose-fed G0 cells (fluorography as in A).

Experiments in A and D were performed by M. Schultz

Experiments in B and C were performed by B. Wu

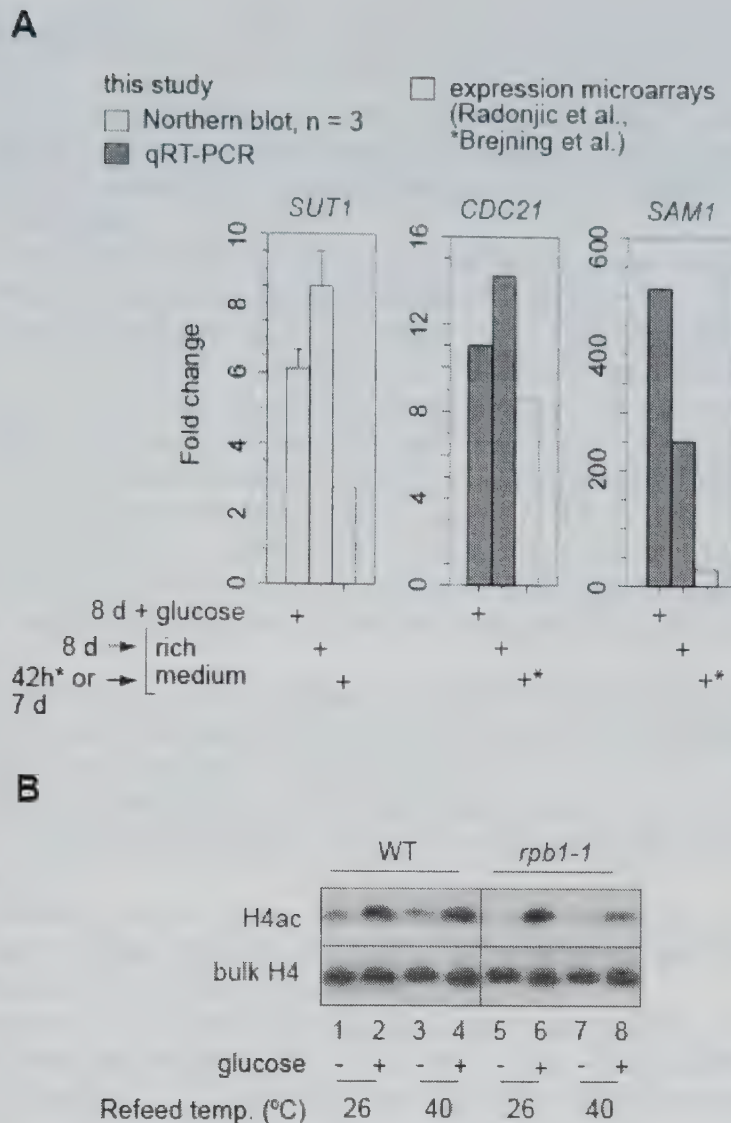


Figure 3.6 Glucose refeed produces a transcriptional response, but ongoing transcription is not required for histone re-acetylation

(A) Glucose induction of transcription in SP cells. Relative abundance of the indicated mRNAs in SP cells at 20 min after refeeding with rich medium containing glucose, or glucose alone. The results obtained by Northern blotting (18S rRNA loading control) and quantitative real-time reverse transcriptase PCR are normalized to expression at time 0. These mRNAs were previously reported to be induced when nutrient-limited cells are resuspended in a large volume of glucose-containing rich medium; this published data (Brejning et al., 2003; Radonjic et al., 2005) is included for comparison. (B) Immunoblotting analysis of H4 acetylation in wild type and *rpb1-1* cells. Cultures (control and glucose-supplemented) were maintained at the permissive or restrictive temperature after glucose addition for 1 hour.

The gene expression analysis in (A) was performed by B.Wu and D.Hockman in consultation with me.

3.4 References

- Adkins, M.W., Carson, J.J., English, C.M., Ramey, C.J. and Tyler, J.K. (2007) The histone chaperone anti-silencing function 1 stimulates the acetylation of newly synthesized histone H3 in S-phase. *J Biol Chem*, **282**, 1334-1340.
- Aggarwal, B.D. and Calvi, B.R. (2004) Chromatin regulates origin activity in *Drosophila* follicle cells. *Nature*, **430**, 372-376.
- Allen, C., Buttner, S., Aragon, A.D., Thomas, J.A., Meirelles, O., Jaetao, J.E., Benn, D., Ruby, S.W., Veenhuis, M., Madeo, F. and Werner-Washburne, M. (2006) Isolation of quiescent and nonquiescent cells from yeast stationary-phase cultures. *J Cell Biol*, **174**, 89-100.
- Antequera, F. (2004) Genomic specification and epigenetic regulation of eukaryotic DNA replication origins. *Embo J*, **23**, 4365-4370.
- Berndsen, C.E., Tsubota, T., Lindner, S.E., Lee, S., Holton, J.M., Kaufman, P.D., Keck, J.L. and Denu, J.M. (2008) Molecular functions of the histone acetyltransferase chaperone complex Rtt109-Vps75. *Nat Struct Mol Biol*.
- Brejning, J., Jespersen, L. and Arneborg, N. (2003) Genome-wide transcriptional changes during the lag phase of *Saccharomyces cerevisiae*. *Arch Microbiol*, **179**, 278-294.
- Cross, F.R., Hoek, M., McKinney, J.D. and Tinkelenberg, A.H. (1994) Role of Swi4 in cell cycle regulation of CLN2 expression. *Mol Cell Biol*, **14**, 4779-4787.
- Drebot, M.A., Veinot-Drebot, L.M., Singer, R.A. and Johnston, G.C. (1990) Induction of yeast histone genes by stimulation of stationary-phase cells. *Mol Cell Biol*, **10**, 6356-6361.
- Ehrenhofer-Murray, A.E. (2004) Chromatin dynamics at DNA replication, transcription and repair. *Eur J Biochem*, **271**, 2335-2349.
- Granot, D. and Snyder, M. (1991) Glucose induces cAMP-independent growth-related changes in stationary-phase cells of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*, **88**, 5724-5728.
- Granot, D. and Snyder, M. (1993) Carbon source induces growth of stationary phase yeast cells, independent of carbon source metabolism. *Yeast*, **9**, 465-479.
- Gray, J.V., Petsko, G.A., Johnston, G.C., Ringe, D., Singer, R.A. and Werner-Washburne, M. (2004) "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev*, **68**, 187-206.
- Kurdistani, S.K. and Grunstein, M. (2003) Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol*, **4**, 276-284.
- Moll, R. and Wintersberger, E. (1976) Synthesis of yeast histones in the cell cycle. *Proc Natl Acad Sci U S A*, **73**, 1863-1867.
- Radonjic, M., Andrau, J.C., Lijnzaad, P., Kemmeren, P., Kockelkorn, T.T., van Leenen, D., van Berkum, N.L. and Holstege, F.C. (2005) Genome-wide analyses reveal RNA polymerase II located upstream of genes poised for rapid response upon *S. cerevisiae* stationary phase exit. *Mol Cell*, **18**, 171-183.
- Ramaswamy, V., Williams, J.S., Robinson, K.M., Sopko, R.L. and Schultz, M.C. (2003) Global control of histone modification by the anaphase-promoting complex. *Mol Cell Biol*, **23**, 9136-9149.

- Rocha, W. and Verreault, A. (2008) Clothing up DNA for all seasons: Histone chaperones and nucleosome assembly pathways. *FEBS Lett*, **582**, 1938-1949.
- Rufiange, A., Jacques, P.E., Bhat, W., Robert, F. and Nourani, A. (2007) Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell*, **27**, 393-405.
- Sandmeier, J.J., French, S., Osheim, Y., Cheung, W.L., Gallo, C.M., Beyer, A.L. and Smith, J.S. (2002) RPD3 is required for the inactivation of yeast ribosomal DNA genes in stationary phase. *Embo J*, **21**, 4959-4968.
- Schwob, E. and Nasmyth, K. (1993) CLB5 and CLB6, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*. *Genes Dev*, **7**, 1160-1175.
- Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T. and Allis, C.D. (1995) Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc Natl Acad Sci U S A*, **92**, 1237-1241.
- Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D. and Futcher, B. (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell*, **9**, 3273-3297.
- van Attikum, H. and Gasser, S.M. (2005) The histone code at DNA breaks: a guide to repair? *Nat Rev Mol Cell Biol*, **6**, 757-765.

Chapter 4: KAT and HDAC control of histone acetylation after glucose refeed

Material in this chapter has been published:

Friis, R.M., Wu, B.P., Reinke, S.N., Hockman, D.J., Sykes, B.D.
and Schultz, M.C. (2009b) A glycolytic burst drives glucose
induction of global histone acetylation by picNuA4 and SAGA.
Nucleic Acids Res, **37**, 3969-3980.

4.1 Introduction

Histone acetylation is mediated by KATs and reversed by HDACs, and controlled to a large extent by mechanisms that impinge on these enzymes. Histone-directed KATs and HDACs, acting mostly as components of heterotypic protein complexes, modify their substrates in the course of targeted and untargeted interactions with chromatin (Friis and Schultz, 2008; Shahbazian and Grunstein, 2007). Untargeted interactions involve soluble KAT and HDAC complexes that function 'randomly' ('globally') throughout chromatin, whereas targeted interactions involve KATs that are recruited to specific locations in chromosomes. The mechanism and functional significance of KAT targeting to gene regulatory regions have been well characterized (Lee and Workman, 2007). To briefly summarize, targeted histone acetylation is typically driven by direct physical interaction of a non-catalytic subunit of a KAT or HDAC complex with an inducible, sequence-specific transcription factor bound to the regulatory region of a gene.

Transcriptional repression by the RPD3L HDAC complex, for example, involves its recruitment to the upstream control regions of genes by sequence-specific transcription factors which

are subunits of RPD3L (Carrozza et al., 2005). Analogous targeting of KATs can aid activation. Tra1, a non-catalytic subunit of some H3 and H4 KAT complexes, directly binds to inducible, sequence-specific transactivators and thereby promotes targeted histone acetylation which favors transcription (Brown et al., 2001).

Studies in budding yeast have revealed that untargeted histone acetylation also contributes to transcriptional regulation (Imoberdorf et al., 2006; Katan-Khaykovich and Struhl, 2002; Kuo et al., 2000; Reid et al., 2000; Vogelauer et al., 2000). Opposing untargeted KAT and HDAC activities generate a default hypoacetylated state that keeps unwanted basal transcription in check. And by catalyzing the rapid turnover of acetyl groups, untargeted KAT/HDAC activities return transcribed genes to a baseline acetylation state when the inducing signal ceases. Untargeted KAT activity may also facilitate transcriptional induction. Experiments using an activator bypass approach suggest that the strength of interaction between an activator and its target in the transcriptional machinery might determine the extent to which induction of natural promoters depends on untargeted acetylation (Imoberdorf et al., 2006). It is important

to note that, while global acetylation by untargeted KATs/HDACs can affect aspects of the transcriptional response, fluctuations in the set point of global acetylation do not drive specific transcriptional responses. Rather, globally acting KATs and HDACs buffer the chromatin environment of genes to an acetylation set-point that is over-written by targeted enzymes during transcriptional induction and repression.

Untargeted KAT activity is critical for normal cell function, as most clearly demonstrated by work on yeast Esa1 (Clarke et al., 1999). This enzyme exists in two complexes, NuA4 and picNuA4, which mainly acetylate H2A and H4 (Boudreault et al., 2003). NuA4 is targeted to genes by the interaction of its Tra1 subunit with activation domains present in sequence-specific DNA-binding transcription factors (Brown et al., 2001). picNuA4 does not contain Tra1 or any other activator binding protein, and therefore is specialized for untargeted acetylation. As cells lacking NuA4 are viable, it appears that the essential functions of *ESA1* are provided by untargeted picNuA4 activity (Boudreault et al., 2003). Untargeted KAT activity may serve important functions in all eukaryotes. For instance, the HBO1 complex in humans is equivalent to picNuA4 in terms of its subunit

composition and specialization for untargeted acetylation (Doyon et al., 2006).

Previous work on the regulation of overall histone acetylation in yeast suggests one obvious and plausible explanation for the mechanism of this regulation. Sandmeier et al. provided compelling evidence that histone deacetylase Rpd3 controls steady state H3/H4 acetylation in SP cells (Sandmeier et al., 2002). It follows that repression of Rpd3 is likely to account for glucose induction of overall acetylation in wild type SP cells. This notion is particularly attractive because it is clear that physiological regulation of Rpd3 targeting can contribute to locus-specific induction of histone acetylation (Sabet et al., 2004; Voth et al., 2007). Surprisingly, our results do not support this model.

Guided by previous studies of KAT complexes, we used a genetic approach to identify KATs that are important for glucose induction of acetylation, the complexes that they act in, and the relative contribution of targeted and untargeted KAT activity to histone acetylation in glucose-fed cells. This work revealed that glucose induction of overall histone acetylation is predominantly

due to the untargeted activities of the KATs Esa1 and Gcn5 acting, respectively, in the picNuA4 and SAGA complexes.

4.2 Results and Discussion

Theoretically, glucose induction of H3/H4 acetylation in SP could be driven by increased KAT activity, decreased HDAC activity, or both. If this regulation is due to events that impinge on an individual KAT or HDAC, then a mutation that compromises the activity of that enzyme should dampen glucose induction of acetylation. We screened candidate KATs and HDACs based on this expectation.

4.2.1 Rpd3 partly controls steady-state H3/H4 acetylation in SP but does not have a major role in glucose induction of acetylation

Among the HDACs that could mediate overall acetylation changes in response to glucose, Rpd3 appeared to be the best candidate. Rpd3 is the catalytic subunit of HDAC complexes that efficiently deacetylate H3 and H4 (Yang and Seto, 2008). moreover, Rpd3 has been implicated in the establishment and/or maintenance of steady state H3/H4 acetylation in SP by the observation that *rpd3Δ* cells have abnormally high histone

acetylation at 4 d in culture (Sandmeier et al., 2003; we also observe induction [2.2-fold] of H4ac in *rpd3Δ* cells at 8 d of culture – Figure 4.1 A, lanes 3, 5). Our finding that Rpd3 expression is as high in SP as in log phase (Figure 4.1 B) increased our expectation that Rpd3 is a critical mediator of glucose induction of histone acetylation. Surprisingly, however, deletion of *RPD3* has only a modest effect on glucose induction of H4ac (for example, 28% lower than wild type in Figure 4.1 A, lanes 3-6). We conclude that glucose-induction of H4 acetylation in SP occurs predominantly by an Rpd3-independent mechanism. Other HDACs were not tested for participation in induction of histone acetylation because, as described below, parallel experiments revealed a major role for KATs in this regulation.

4.2.2 Histone lysine acetylases Esa1 and Gcn5 mediate glucose induction of H3/H4 acetylation in SP

We predicted an important role for Esa1 in the regulation of H4ac in SP as this KAT is required for maintenance H4 acetylation in log phase (Clarke et al., 1999) and is expressed in SP (Figure 4.2 A). Because Esa1 is essential for viability, its function was tested by analysis of two temperature sensitive

mutants (Clarke et al., 1999) that were allowed to reach SP at the permissive temperature.

Figure 4.2 B shows that Esa1 activity is required for maintenance of basal acetylation in SP. At room temperature *esa1-414* protein is partially inactive (Allard et al., 1999), and *esa1-414* cells display lower than wild type H4 acetylation (lanes 1, 3). Furthermore, H4 acetylation drops in *esa1-414* and *esa1-L254P* mutants when they are shifted to the restrictive temperature in SP (Figure 4.2 B, lanes 3, 9; 5, 11), but does not do so in similarly-treated wild type cells (Figure 4.2 B, lanes 1, 7). Accordingly, we propose that dynamic H4 acetylation in SP directly involves Esa1.

A central role for Esa1 in glucose induction of H4ac in SP was uncovered by refeeding at the permissive and restrictive temperatures. At the permissive temperature, induction of H4 acetylation was slower in *esa1^{ts}* mutants (particularly *esa1-414*) than in wild type cells (Figure 4.2 B, lanes 1-4). When cells grown for 8 d at the permissive temperature were incubated for 1 h at the restrictive temperature and then fed at the restrictive temperature, there was an even more pronounced effect specifically in *esa1^{ts}* mutants: H4 acetylation was almost

completely blocked (Figure 4.2 B, lanes 7-12). On the other hand, null mutants of *HAT2* and *SAS2*, which encode the other two major H4 KATs in yeast, induce H4ac normally (Figure 4.2 C). We conclude that Esa1 activity is critical for robust glucose induction of H4 acetylation in SP. Esa1 does not contribute to H3 acetylation in log phase cells (Suka et al., 2001), and is not important for glucose induction of H3ac in SP (Figure 4.2 D).

Of the known H3 KATs, Gcn5 was expected to play an important role in the regulation of H3ac in SP because it has long been known to contribute to the control of overall acetylation of H3 K9 in log phase (Howe et al., 2001; Suka et al., 2001). Indeed, H3 acetylation in 8 d *gcn5Δ* cells is much lower than in wild type cells, and *gcn5Δ* do not regain the wild type level of H3ac after glucose refeed (Figure 4.3 A, lanes 1-4). On the other hand, *GCN5* deletion has little effect on induction of H4ac (Figure 4.3 B), and the regulation of H3 acetylation is not perturbed by deletion of two other H3 KATs (Hpa2 and Sas3; Figure 4.3 A, lanes 5-8). We conclude that Gcn5 functions in the establishment and/or maintenance of global H3 acetylation in SP, and has an important role in induction of H3ac upon glucose refeeding.

One could argue, because *esa1* and *gcn5* mutants have a lower level of acetylation in SP than wild type cells and fail to induce acetylation to wild type levels when fed glucose (Figures 4.2 B, 4.3 A), that induction of acetylation in SP depends on Esa1/Gcn5 solely for establishment of a threshold level of acetylation that is permissive for glucose induction by other KATs. We do not favour this scenario because glucose induction of acetylation is normal in a non-KAT mutant that has low acetylation in SP (*rpb1-1*, Figure 3.6 B).

Our results do not exclude the possibility that glucose induction of H3/H4 acetylation in SP cells partly involves KATs in addition to Gcn5 and Esa1. Given the evident importance of Gcn5 and Esa1 in this regulation, however (Figure 4.2 B, 4.3 A), our further work focused on these enzymes.

4.2.3 Glucose induction of histone acetylation by Esa1 and Gcn5 in SP is predominantly due to the untargeted activities of picNuA4 and SAGA

Overall histone acetylation is a combined readout of targeted (de)acetylation events that are directly coupled to transcriptional reprogramming, and untargeted (de)acetylation

events that modulate global acetylation in the absence of recruited KAT/HDAC activities. The KATs responsible for H3/H4 acetylation in response to glucose, Esa1 and Gcn5, both function in targeted and untargeted acetylation, and both exist in multiple complexes that acetylate chromatin (Friis and Schultz, 2008; Lee and Workman, 2007). We used a genetic approach to assess the relative contribution of targeted and untargeted acetylation to induction of overall acetylation, and to identify the KAT complexes responsible for histone acetylation upon glucose refeeding.

Esa1 occurs in one complex (NuA4) which functions in targeted acetylation and another (picNuA4) which is specialized for untargeted acetylation (Boudreault et al., 2003). We differentiated the activities of NuA4 and picNuA4 by monitoring the glucose response of a viable mutant that lacks NuA4 activity because its Epl1 subunit is expressed in a C-terminally truncated form (Boudreault et al., 2003). *epl1(1-485)* is present in SP at levels comparable to Epl1 (Figure 4.4, A) and *epl1(1-485)* cells support robust glucose induction of H4ac (Figure 4.4, B). Therefore, although Esa1 is required for H4 acetylation triggered by glucose (Figure 4.2, B), NuA4 is not. Accordingly we propose

that glucose induction of H4 acetylation in SP is mainly due to untargeted Esa1 activity.

The Esa1-dependent picNuA4 complex likely performs untargeted acetylation of nucleosomal H4 in glucose-fed cells, since it acetylates nucleosomal histones more readily than it does free histones, and it lacks the Tra1 targeting protein (Boudreault et al., 2003). Also of note, the cellular yield of picNuA4 increases with culture density (Boudreault et al., 2003), and its Esa1, Epl1 and Yng2 subunits are expressed in SP (Figures 4.2 A, 4.4 A, data not shown).

Although Gcn5 can acetylate nucleosomes on its own (Tse et al., 1998), in yeast this enzyme mostly exists in complex with other proteins (Lee and Workman, 2007). Therefore we predicted that a Gcn5 complex performs glucose-induced H3 acetylation. Our first step in testing this prediction was to comprehensively analyze the site-specificity of Gcn5-dependent H3 tail acetylation in glucose-fed cells. For this experiment blots were probed with antibodies which exclusively recognize H3 K9ac, H3K14ac, H3K18ac and H3K27ac, as well as an antibody directed against acetyl-lysine (Kac). As noted earlier (Figure 3.2 D), acetylation of H3 K9, 14, 18 and 27 is induced by glucose

(Figure 4.5 A, lanes 1, 2). Furthermore, as expected from published studies of the KAT-dependency of H3 tail acetylation in log phase cells (Durant and Pugh, 2006; Peng et al., 2008; Suka et al., 2001) , acetylation of H3 K9, K18 and K27, but not K14, strongly depends on Gcn5 (Figure 4.5 A, lanes 3, 4).

Three complexes which contain Gcn5 and can acetylate nucleosomal substrates have been well characterized. These are SAGA, SLIK and ADA. We tested if proteins required for the stability of such complexes are required for H3 acetylation in response to glucose. SAGA and SLIK are not detectable in cells lacking Spt7, *rtg2Δ* cells lack SLIK but not SAGA (Pray-Grant et al., 2005), and the ADA complex is absent in cells lacking Ahc1 (Eberharther et al., 1999). We find that glucose induction of Gcn5-dependent H3 acetylation is completely blocked in *spt7Δ* cells, but unaffected in *AHC1* and *RTG2* null mutants (Figure 4.5 A, B). This profile suggests that SAGA is sufficient for glucose induction of H3 K9, K18, K27 acetylation, and that neither ADA nor SLIK on its own is 'necessary and sufficient' for the response. Deletion of *SPT8*, which interferes with many known functions of SAGA (Pray-Grant et al., 2005), does not block glucose induction of H3 acetylation (Figure 4.5 B). Induction of acetylation

therefore is either separable from Spt8-dependent functions of SAGA, or can also be mediated by SLIK. A straightforward and parsimonious interpretation of these results is that SAGA is sufficient for glucose-induction of H3 acetylation by Gcn5. An alternative possibility is that an unknown SAGA-like complex mediates untargeted H3 acetylation in glucose-fed cells. We do not favour this possibility because intensive characterization of Gcn5-dependent KATs by multiple independent groups has failed to reveal Spt7-dependent Gcn5 complexes in addition to SAGA (Grant et al., 1997; Lee and Workman, 2007; Pray-Grant et al., 2002; Sterner et al., 2002).

We tested if Gcn5 targeting is important for glucose induction of H3 acetylation using strains harbouring mutations in known targeting subunits of SAGA/SLIK. These are Tra1, which mediates association of SAGA/SLIK with trans-activators, and the chromodomain protein Chd1 which binds to K4-methylated H3 in nucleosomes (Pray-Grant et al., 2005). *tra1-2* cells (Brown et al., 2001) acetylate H3 normally in response to glucose at the restrictive temperature (Figure 4.6 A), as do *chd1Δ* cells and a *tra1-2 chd1Δ* double mutant at the restrictive temperature (Figure 4.6 B). It follows that neither Tra1 nor

Chd1 plays a substantial role in overall acetylation of H3 after glucose refeeding in SP. Accordingly, we propose that SAGA-dependent H3 acetylation in glucose-fed cells occurs by an untargeted mechanism. Furthermore, since inactivation of Tra1 prior to refeeding does not dampen glucose induction of H3 acetylation (Figure 4.6 B), we consider it unlikely that H3 acetylation is due to passive spreading of targeted H3 KATs from induced genes (Howe et al., 2001).

We do not exclude glucose induction of targeted Esa1 or Gcn5 activity in SP cells. It is clear from our results, however, that induction of targeted activity makes only a minor contribution to glucose induction of overall Esa1/Gcn5-dependent acetylation in the amino-terminal tails of H3 and H4.

4.3 Conclusions

The increases in overall histone acetylation which are attendant upon glucose refeed appear to be mediated by untargeted KAT activities. Targeted recruitment of KATs is certain to occur under these circumstances but large changes in the genome wide global acetylation level are likely to overshadow these local targeted changes. Modulation of global

histone acetylation in response to availability of glucose could aid in the general regulation of transcription in times of “boom or bust”. In SP energy is limiting, and lower global acetylation levels facilitate conservative management of energy-demanding transcriptional processes. Emphasis is put on preventing transcription that is not strictly needed (by increasing the strength of activation signal required for initiation of transcription) and rapidly returning genes to the repressed state when their transcription is no longer needed. The increased global acetylation level after glucose refeed represents a shift in focus. Glucose provides a “fed” signal and cells strive to achieve rapid assimilation of available nutrients. High global acetylation favors the transcriptional on state by lowering the threshold for activation of transcription. This promotes anabolic activity at the expense of conservative energy management. Previous studies of global histone acetylation have modulated global histone acetylation levels by deleting KATs or HDACs that have untargeted activities. The physiological regulation of untargeted activities, shown here, demonstrates that the transcriptional effects observed in studies which have artificially manipulated global acetylation are significant *in vivo*.

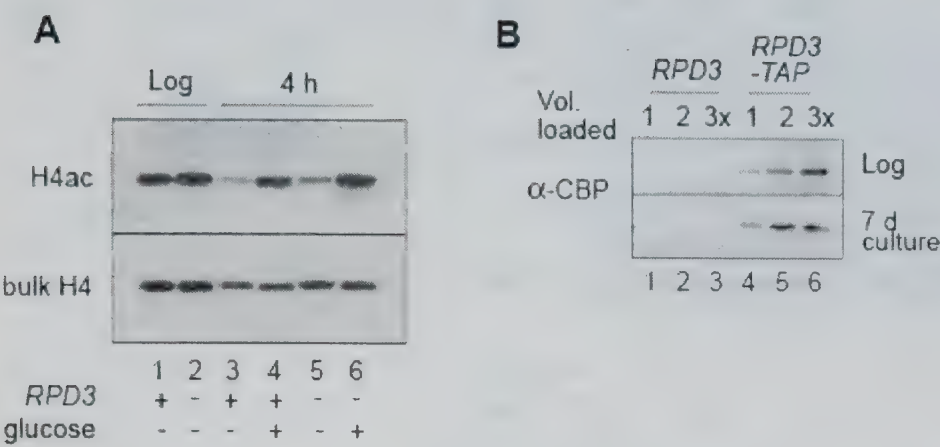


Figure 4.1 Rpd3 protein expression in SP and effect of *RPD3* deletion on H4 acetylation.

(A) Immunoblotting analysis of H4 acetylation in wild type (*RPD3* +) and *rp d3Δ* cells (*RPD3* -), during active proliferation (log) and in SP (at 4 h after glucose feeding or no refeeding).

(B) Expression of Rpd3-TAP in actively growing (log) and SP (7 d culture) cells monitored by anti-CBP immunoblotting. Lysate from wild type cells (no tagged protein) served as a specificity control.

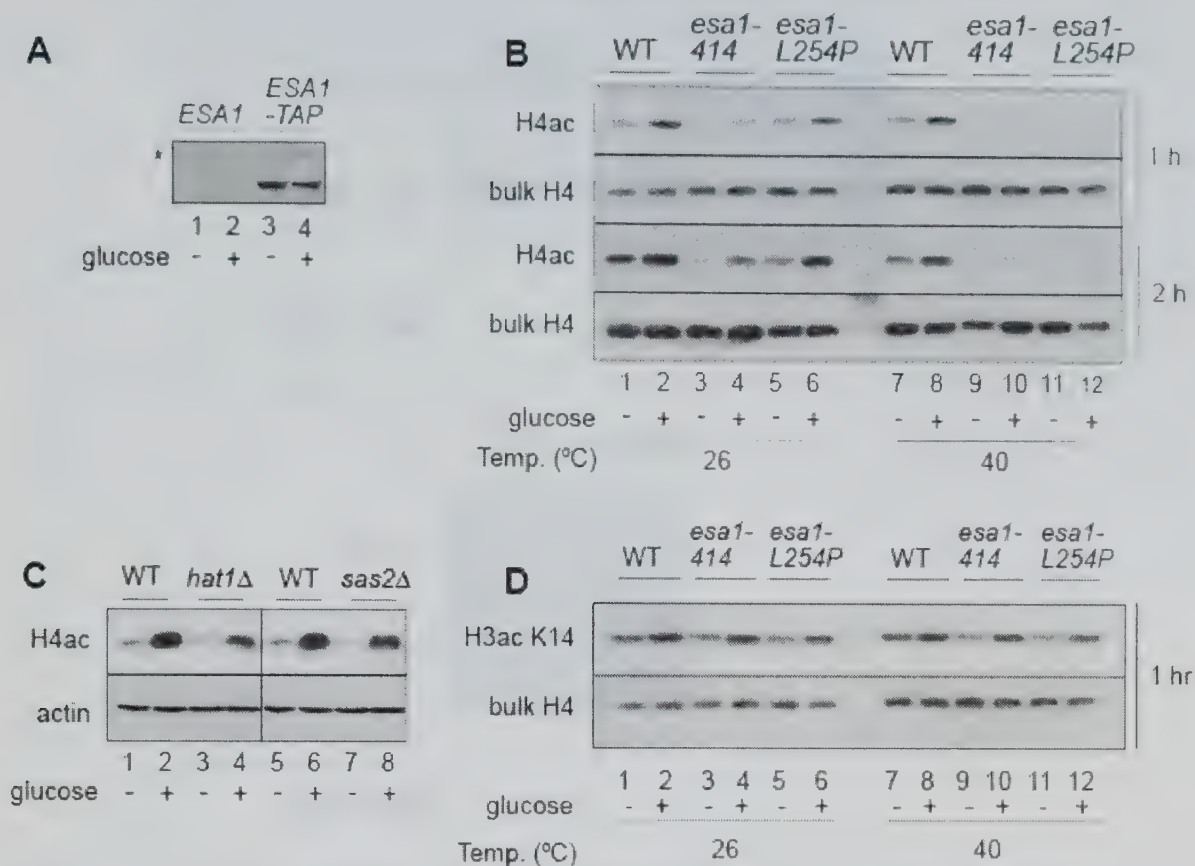


Figure 4.2 Identification of the KATs required for glucose induction of H4 acetylation in SP.

(A) Expression of Esa1-TAP in unfed and glucose-fed (1h) SP cells monitored by anti-CBP immunoblotting. Equivalent loading is confirmed by the intensity of the background band (*).

(B) Immunoblotting analysis of H4 acetylation in wild type and two *ESA1* temperature sensitive strains. Cells were unfed or glucose-fed, at either the permissive (26°C) or restrictive (40°C) temperature.

(C) Immunoblotting analysis of H4 acetylation in wild type and *HAT1* and *SAS2* null mutants. Glucose-fed cells were harvested at 1 h.

(D) Immunoblotting analysis of H3 K14 acetylation in wild type and *ESA1* mutant cells.



Figure 4.3 Identification of the KATs required for glucose induction of H3 acetylation in SP.

(A) Immunoblotting analysis of H3 acetylation in wild type and *GCN5*, *HPA2* and *SAS3* null mutants. Glucose-fed cells were harvested at 1 h. Glucose was added to 2% weight/volume for all experiments.

(B) Immunoblotting analysis of H4 acetylation in wild type and *gcn5Δ* cells. The loading control is actin.

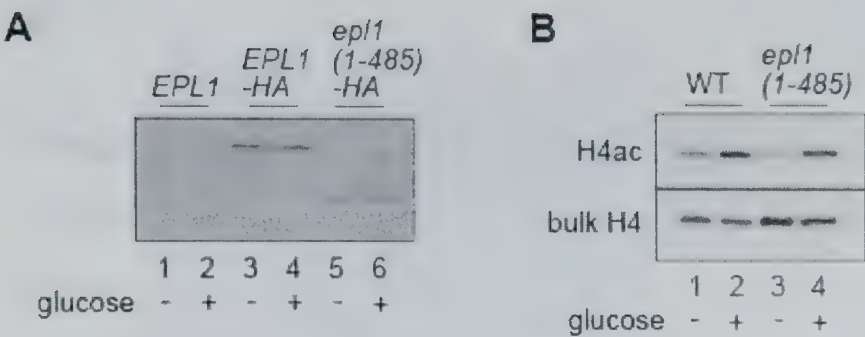


Figure 4.4 Identification of H4 KAT complex required for glucose induction of H4 acetylation in SP.

(A) Expression of TAP-tagged Epl1 and epl1(1-485) monitored by anti-CBP immunoblotting. Glucose-fed and un-fed cells were harvested at 1 h.

(B) Immunoblotting analysis of H4 acetylation in wild type and *epI1*(1-485) cells. Glucose-fed and un-fed cells were harvested at 1 h

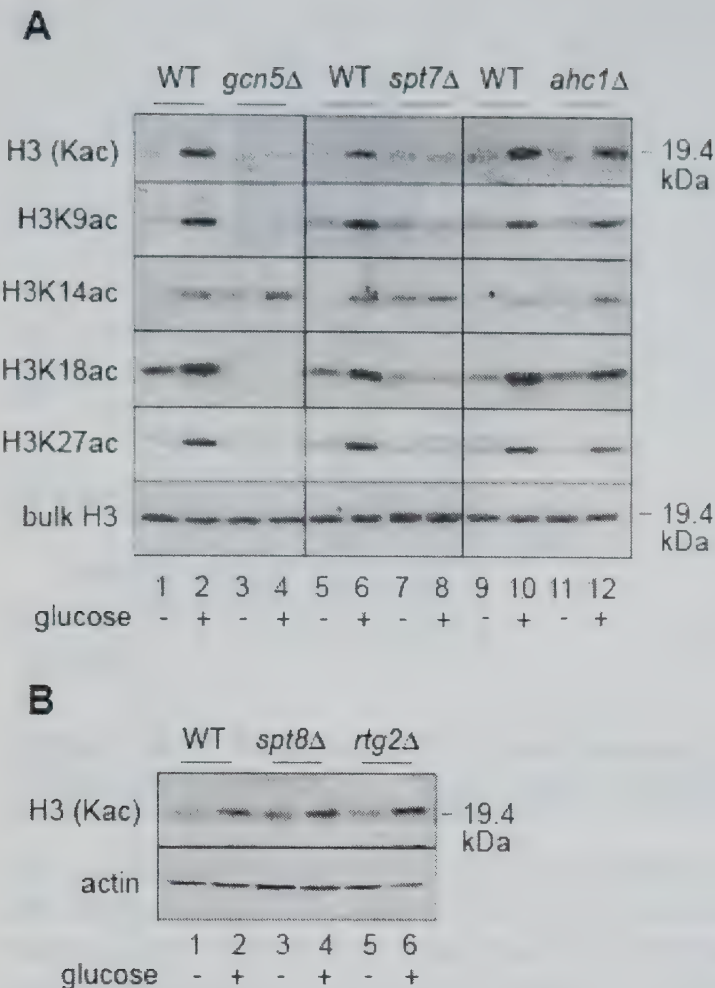


Figure 4.5 Identification of H3 KAT complex required for glucose induction of H3 acetylation in SP.

(A) Immunoblotting analysis of H3 acetylation in wild type cells compared to null mutants lacking the genes encoding Spt7 (specific for SAGA/SLIK) and Ahc1 (a specific subunit of ADA). Glucose-fed and un-fed cells were harvested at 1 h.

(B) Immunoblotting analysis of H3 acetylation in wild type and null mutant strains lacking the genes encoding Spt8 (a specific subunit of SAGA) and Rtg2 (a specific subunit of SLIK). Glucose-fed and un-fed cells were harvested at 1 h.

Acetylated H3 was detected using an anti-acetyl lysine antibody (Kac).

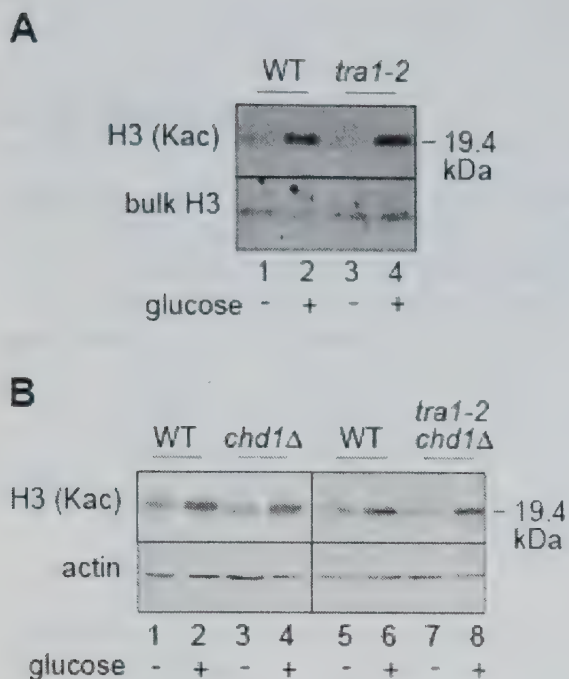


Figure 4.6 Analysis of involvement of targeting subunits of SAGA in glucose induction of H3 acetylation in SP.

(A) Immunoblotting analysis of H3 acetylation in wild type and a *TRA1* temperature sensitive strain. Cells were incubated for 30 min at the restrictive temperature (40°C), then harvested after another 1 h at the same temperature in the presence or absence of added glucose. Acetylated H3 was detected using an anti-acetyl lysine antibody (Kac).

(B) Immunoblotting analysis of H3 acetylation in wild type and mutant strains lacking *CHD1* in a *TRA1* or *tra1-2* background. The *chd1Δ* single mutant was fed at room temperature and harvested at 1 h. The double mutant was fed after 30 min at the restrictive temperature (40°C) and harvested 1 h later. Acetylated H3 was detected using an anti-acetyl lysine antibody (Kac).

4.4 References

- Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L., Workman, J.L. and Cote, J. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *Embo J*, **18**, 5108-5119.
- Boudreault, A.A., Cronier, D., Selleck, W., Lacoste, N., Utley, R.T., Allard, S., Savard, J., Lane, W.S., Tan, S. and Cote, J. (2003) Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev*, **17**, 1415-1428.
- Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S. and Workman, J.L. (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science*, **292**, 2333-2337.
- Carrozza, M.J., Florens, L., Swanson, S.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005) Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. *Biochim Biophys Acta*, **1731**, 77-87; discussion 75-76.
- Clarke, A.S., Lowell, J.E., Jacobson, S.J. and Pillus, L. (1999) Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol Cell Biol*, **19**, 2515-2526.
- Doyon, Y., Cayrou, C., Ullah, M., Landry, A.J., Cote, V., Selleck, W., Lane, W.S., Tan, S., Yang, X.J. and Cote, J. (2006) ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Mol Cell*, **21**, 51-64.
- Durant, M. and Pugh, B.F. (2006) Genome-wide relationships between TAF1 and histone acetyltransferases in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **26**, 2791-2802.
- Eberharter, A., Sterner, D.E., Schieltz, D., Hassan, A., Yates, J.R., 3rd, Berger, S.L. and Workman, J.L. (1999) The ADA complex is a distinct histone acetyltransferase complex in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **19**, 6621-6631.
- Friis, R.M.N. and Schultz, M.C. (2008) Untargeted tail acetylation of histones in chromatin: lessons from yeast. *Biochem Cell Biol*, **in press**.
- Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev*, **11**, 1640-1650.
- Howe, L., Auston, D., Grant, P., John, S., Cook, R.G., Workman, J.L. and Pillus, L. (2001) Histone H3 specific acetyltransferases are essential for cell cycle progression. *Genes Dev*, **15**, 3144-3154.
- Imoberdorf, R.M., Topalidou, I. and Strubin, M. (2006) A role for gcn5-mediated global histone acetylation in transcriptional regulation. *Mol Cell Biol*, **26**, 1610-1616.
- Katan-Khaykovich, Y. and Struhl, K. (2002) Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal

- histone acetylation status upon removal of activators and repressors. *Genes Dev*, **16**, 743-752.
- Kuo, M.H., vom Baur, E., Struhl, K. and Allis, C.D. (2000) Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. *Mol Cell*, **6**, 1309-1320.
- Lee, K.K. and Workman, J.L. (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol*, **8**, 284-295.
- Peng, W., Togawa, C., Zhang, K. and Kurdistani, S.K. (2008) Regulators of cellular levels of histone acetylation in *Saccharomyces cerevisiae*. *Genetics*, **179**, 277-289.
- Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd and Grant, P.A. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature*, **433**, 434-438.
- Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R., 3rd and Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol Cell Biol*, **22**, 8774-8786.
- Reid, J.L., Iyer, V.R., Brown, P.O. and Struhl, K. (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol Cell*, **6**, 1297-1307.
- Sabet, N., Volo, S., Yu, C., Madigan, J.P. and Morse, R.H. (2004) Genome-wide analysis of the relationship between transcriptional regulation by Rpd3p and the histone H3 and H4 amino termini in budding yeast. *Mol Cell Biol*, **24**, 8823-8833.
- Sandmeier, J.J., French, S., Osheim, Y., Cheung, W.L., Gallo, C.M., Beyer, A.L. and Smith, J.S. (2002) RPD3 is required for the inactivation of yeast ribosomal DNA genes in stationary phase. *Embo J*, **21**, 4959-4968.
- Shahbazian, M.D. and Grunstein, M. (2007) Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem*, **76**, 75-100.
- Sterner, D.E., Belotserkovskaya, R. and Berger, S.L. (2002) SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription. *Proc Natl Acad Sci U S A*, **99**, 11622-11627.
- Suka, N., Suka, Y., Carmen, A.A., Wu, J. and Grunstein, M. (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. *Mol Cell*, **8**, 473-479.
- Tse, C., Georgieva, E.I., Ruiz-Garcia, A.B., Sendra, R. and Hansen, J.C. (1998) Gcn5p, a transcription-related histone acetyltransferase, acetylates nucleosomes and folded nucleosomal arrays in the absence of other protein subunits. *J Biol Chem*, **273**, 32388-32392.
- Vogelauer, M., Wu, J., Suka, N. and Grunstein, M. (2000) Global histone acetylation and deacetylation in yeast. *Nature*, **408**, 495-498.
- Voth, W.P., Yu, Y., Takahata, S., Kretschmann, K.L., Lieb, J.D., Parker, R.L., Milash, B. and Stillman, D.J. (2007) Forkhead proteins control the outcome of transcription factor binding by antiactivation. *Embo J*, **26**, 4324-4334.
- Yang, X.J. and Seto, E. (2008) The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nat Rev Mol Cell Biol*, **9**, 206-218.

Chapter5: Analysis of cell signaling and metabolic effects on histone acetylation

Material in this chapter has been published:

Friis, R.M., Wu, B.P., Reinke, S.N., Hockman, D.J., Sykes, B.D. and Schultz, M.C. (2009b) A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. *Nucleic Acids Res*, **37**, 3969-3980.

5.1 Introduction

Glucose induction of H3/H4 acetylation largely depends on two KATs which play a pivotal role in transcription in yeast and higher eukaryotes (Avvakumov and Cote, 2007; Durant and Pugh, 2006; Lafon et al., 2007; Nagy and Tora, 2007). These are Gcn5, which acetylates H3, and Esa1, which acetylates H4 (Millar and Grunstein, 2006). Surprisingly, however using genetic manipulations to affect targeting of Gcn5 and Esa1, we have demonstrated that these KATs can mediate glucose induced histone acetylation in an untargeted fashion. The picNuA4 complex which contains Esa1 but does not interact with activator proteins appears to mediate the acetylation of H4. The acetylation on H3 depends upon the Gcn5 containing SAGA complex, but does not depend on the activities of the SAGA component Tra1 which is responsible for targeting of SAGA.

In theory, the same signalling pathways which initiate the transcriptional response to glucose could induce untargeted KAT activities. Approximately 90% of the transcriptional response to glucose seems to result from the induction of protein kinase A (PKA) activity (Wang et al., 2004; Zaman et al., 2009). Induction of PKA activity is achieved when the two regulatory

subunits of a PKA heterotetramer bind cAMP and disassociate from the catalytic subunits (Toda et al., 1987). Glucose stimulates production of cAMP by the adenylate cyclase Cyc1/Cdc35. This occurs via activation of the small GTP binding proteins Ras1, Ras2 and Gpa2, any of which can bind to and activate Cdc35 when in their GTP bound active form. Gpa2 interacts with the G protein coupled receptor Gpr1. Binding of glucose to Gpr1 is thought to stimulate GTP binding by Gpa2 (Lemaire et al., 2004). Although the mechanism remains unclear, glucose promotes activation of the Ras proteins through the activity of the GTP exchange factor Cdc25. The TOR protein kinases, Tor1 and Tor2, are also responsive to nutrient cues and can control many of the same genes affected by PKA signalling through activation of Sch9 (Urban et al., 2007; Zaman et al., 2009). Phosphorylation of the Sch9 C-terminal end, associated with induction of its protein kinase activity, can be induced by refeeding glucose to glucose-deprived cells. Overexpression of Sch9 mimics the transcriptional effects of PKA activation. However, PKA appears to be sufficient to mediate glucose regulation of these genes alone. Deletion of *SCH9* does not attenuate the strength of the transcriptional response to glucose (Zaman et al., 2009). Interestingly, simultaneous inhibition of

Sch9 and PKA during glucose feeding blocks the transcriptional response of PKA-regulated genes whereas inhibition of PKA alone only reduces the magnitude of this response to one quarter of that seen without perturbation (Zaman et al., 2009).

In contrast to targeted acetylation, where signalling pathways impinge upon transcriptional activators and repressors to subsequently recruit KAT or HDAC complexes, a signalling pathway must directly affect the abundance, localization, or activity of untargeted enzymes to affect untargeted acetylation. Under certain circumstances, transcriptional control of the abundance of an untargeted activity might influence the global acetylation level. Glucose-induced increase in acetylation can occur without the targeting of KAT complexes which would facilitate new transcription. Moreover, the glucose-induced increase in acetylation is not blocked by inhibition of transcription. Therefore, upregulation of transcription of the KATs involved in the response to glucose is unlikely to play a dominant role (Figures 3.6 B; 4.4 B and 4.6).

Abundance of a factor involved in untargeted acetylation might also be regulated by protein turnover. For example, the HDAC Hst3 is phosphorylated in response to genotoxic stress.

This phosphorylation, which depends upon signalling by Mec1, results in ubiquitin mediated proteolysis of Hst3 (Thaminy et al., 2007).

Mammalian studies have revealed direct modulation of KAT and HDAC activities by various pathways. The activity of mammalian KAT p300 is stimulated by Akt-mediated phosphorylation at S1834 of p300, and inhibited by protein kinase C δ phosphorylation at S89 (Huang and Chen, 2005; Yuan et al., 2002). Additionally, phosphorylation of Tip60 and hGcn5, the mammalian homologues of Esa1 and Gcn5, respectively, affects their activity. Tip60 activity is stimulated by its phosphorylation by the cyclinB/Cdc2 complex (Lemerrier et al., 2003), whereas the activity of hGcn5 is inhibited by DNA-PK mediated phosphorylation (Barlev et al., 1998). Signalling pathways also impinge upon the nuclear localization of enzymes. Phosphorylation of human HDAC5 at S259 and S498 by the AMP-sensitive kinase allows 14-3-3 proteins to associate with HDAC5 leading to its nuclear export (McGee et al., 2008). Interestingly, both Esa1 and Rpd3 interact with the yeast 14-3-3 proteins Bmh1 and Bmh2 (Lottersberger et al., 2007). The effects mediated by these 14-3-3 proteins seem to potentiate histone

acetylation. Heat inactivation of temperature sensitive *bmh* mutants results in decreased levels of overall histone acetylation on H4 K5 and H3 K14 (Lottersberger et al., 2007). H3 K14 can be acetylated by Esa1 *in vitro* (Clarke et al., 1999; Smith et al., 1998). These types of signalling events all have the capability to modulate the activities of KAT and HDAC enzymes, and could potentially be involved in the regulation of global histone acetylation.

Alternatively, changes in intracellular concentrations of various metabolites of glucose might also affect the activities of untargeted enzymes. Metabolic control of histone acetylation has been of great interest in biology since biochemical studies revealed that a key small molecule intermediate in metabolism, NAD^+ , is an essential cofactor for the deacetylation reaction catalyzed by some HDACs (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). Based partly on this finding, it was proposed that fluctuations in carbon metabolism could directly underlie fluctuations in histone acetylation (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). Indeed, it is now generally accepted that changes in carbohydrate metabolism can underlie physiological regulation of histone acetylation at lysines

acted on by NAD^+ -dependent HDACs (Grubisha et al., 2005). Current models in yeast for example propose that moderate caloric restriction (glucose limitation) induces NAD^+ -dependent HDACs by increasing the $\text{NAD}^+:\text{NADH}$ ratio in the cell (Chen and Guarente, 2007).

The concept that metabolic mechanisms can control histone acetylation has recently been extended to KATs by the observation that artificial depletion of acetyl-CoA, an essential co-substrate for all KAT reactions, causes dramatic loss of histone acetylation in budding yeast (Takahashi et al., 2006). Takahashi and colleagues proposed an important model in which the steady state level of histone tail acetylation is partly controlled by a metabolic mechanism that dictates the availability of acetyl-CoA for use by nuclear KATs (Fig. 6C in Takahashi et al. 2006). Since carbon metabolism fluctuates, one implicit prediction of this model is that physiological variations in histone acetylation might be regulated by changes in the availability of acetyl-CoA. It remains to be shown, however, that physiological changes in carbon metabolism actually can control fluctuations in the level of KAT activity or the availability of acetyl-CoA for histone acetylation.

The experiments described in this chapter examine both signalling and metabolic control of histone acetylation after glucose refeed. We examine the effects of inhibition of components of major nutrient sensitive signalling pathways, and genetic and pharmacological perturbation of carbon metabolism, and detail the metabolic profile of WT cells during glucose refeed using enzymatic methods and ^1H -NMR. This approach revealed that glucose induction of KAT activity directly depends on increased glucose catabolism.

5.2 Results and Discussion

5.2.1 Perturbation of two major glucose signaling modules does not interfere with glucose induction of histone acetylation in SP

Two distinct regulatory mechanisms that could contribute to glucose induction of untargeted KATs were investigated. Much of cellular regulation depends on signal transduction cascades, we therefore tested if signaling previously implicated in gene induction by glucose contributes to the control of untargeted KAT activity. Protein kinase A plays a critical role in the response of cells to glucose (Zaman et al., 2008). The predominant pathway for glucose activation of PKA involves the GTP-binding Ras

proteins (Figure 5.1 A). Inactivation of Cdc25, the guanine nucleotide exchange factor for Ras, compromises glucose induction of PKA but not glucose induction of H4 acetylation (Figure 5.1 B). Glucose also activates PKA by a pathway that includes the glucose transporter Gpr1 and the small GTP-binding protein Gpa2 (Figure 5.1 A). Inactivation of the Gpr1/Gpa2 circuit in *gpr1Δ* and *gpa2Δ* cells however does not perturb glucose induction of H4 acetylation (Figure 5.1 C). Therefore, either activation of PKA is not 'necessary and sufficient' for glucose induction of H4 acetylation, or sufficient PKA activation can occur through either Ras or Gpa2. Ras- and Gpa2-dependant activation of PKA by glucose depends on cAMP synthesis by Cyr1 (Cdc35). Since inactivation of Cyr1/Cdc35 does not block glucose induction of histone acetylation (Figure 5.1 D), we conclude that PKA signalling is not essential for this regulation.

Glucose also triggers PKA-independent signalling involving the target of rapamycin protein kinases Tor1 and Tor2, acting as components of TOR Complex 1 (TORC1 in Figure 5.1 A) (De Virgilio and Loewith, 2006; Zaman et al., 2008). Glucose may control TORC1 activity through inactivation of Snf1 which

phosphorylates the TORC1 component Kog1 in the absence of glucose (Dechant and Peter, 2008). The TORs are specifically and potently inhibited by the macrolide antibiotic rapamycin. We tested if TOR signalling contributes to glucose induction of H4 acetylation in SP by treating cells with drug vehicle or rapamycin for 30 min prior to glucose addition. Immunoblotting revealed little or no effect of rapamycin on the accumulation of acetylated H4 during the 4 h following glucose refeeding (Figure 5.2 A, lanes 1-12).

This negative result does not reflect the failure of rapamycin to inhibit TOR function in SP cells. Figure 5.2 A shows that at 24 and 48 h after refeeding, the recovery of acetylated H4 increases in proportion to the concentration of rapamycin used to treat the cells (lanes 13-20). Thus, rapamycin inhibits H4 deacetylation in SP cells. Normal H4 deacetylation could be inhibited in treated cells because rapamycin interferes with glucose uptake and therefore prolongs exposure to the inducing signal; the latter would perhaps be equivalent to supplementation with 10% glucose (Figure 5.2 B). This possibility however seems unlikely because glucose is removed from the medium at the same rate in control and rapamycin-treated cultures (Figure 5.2 B).

Finally, because rapamycin inhibition of H4 deacetylation depends on the specific receptor for rapamycin, Fpr1 (Heitman et al., 1991), it is not an off-target effect of the drug. This dependency is evident in Figure 5.2 C; at 24 h after refeeding, for example, H4 acetylation is higher in rapamycin-treated cells only if they express Fpr1 (compare *FPR1* in lanes 13 and 14 to *fpr1Δ* in lanes 15 and 16). Since rapamycin exerts its effect on H4 deacetylation by inhibiting the TORs, we propose that its failure to inhibit glucose induction of H4ac reflects the fact that TOR signalling is not necessary and sufficient for this regulation. Our results do not rule out the possibility that redundant signalling pathways are necessary for glucose induction of histone acetylation.

The activity of Sch9 is regulated by TORC1. As considerable overlap in the functions of Sch9 and PKA exists (Zaman et al., 2009). Both PKA and Sch9 activity can be stimulated by glucose (Urban et al., 2007). Therefore, activity of either Sch9 or PKA might be sufficient to induce histone acetylation in response to glucose refeed. We analyzed this possibility by simultaneously blocking PKA and Sch9 activity during glucose refeed. For this purpose we used the *pka^{as}sch9^{as}* strain developed in the Broach

lab (Zaman et al., 2009). In this strain, all 3 PKA genes (*TPK1*, *TPK2* and *TPK3*) and *SCH9* carry mutations that allow them to be inhibited by the cell permeable ATP analogue C3-1'-naphthyl-methyl PP1 (1NM-PP1). A large side group on 1NM-PP1 prevents it from interacting with wild type kinases thereby allowing specific inhibition of PKA and Sch9 in this strain (Zaman et al., 2009). Thirty minutes before a normal glucose refeed, 1NM-PP1 or an equivalent volume of its vehicle (DMSO) were added to cultures of WT and *pka^{as}sch9^{as}* cells. The presence of 1NM-PP1 did not inhibit the histone acetylation which occurs after glucose in WT or *pka^{as}sch9^{as}* cells (Figure 5.3). Therefore, histone acetylation after glucose refeed does not depend upon either of these kinases.

5.2.2 A metabolic mechanism for glucose induction of global histone acetylation

Based on the idea that increased synthesis of acetyl-CoA, an essential co-substrate of KATs, could stimulate acetylation, we also tested if glucose metabolism is necessary for upregulation of overall histone acetylation by glucose. This idea builds on the following published results. First, artificial depletion of nuclear acetyl-CoA in log phase cells causes overall histone

acetylation to drop precipitously (Takahashi et al., 2006).

Second, acetyl-CoA essentially disappears from cells in early SP (Seker et al., 2005). And finally, cells that have depleted glucose from the medium (1 d culture) can synthesize acetate and (by inference) ATP when fed glucose (Pedler et al., 1997). As noted by Takahashi et al., Acs1 and Acs2 receive substrates from glycolytic metabolism (Takahashi, McCaffery et al. 2006). Since glycolytic activity can fluctuate during the life history of a cell, physiological variations in histone acetylation might be regulated by changes in the availability of acetyl-CoA which result directly from changes in glycolytic rate. Accordingly we envisioned that acetate and/or ATP resulting from glucose metabolism would be used by acetyl-CoA synthases to increase the availability of acetyl-CoA for histone acetylation by untargeted KATs (Kresnowati et al., 2006).

Our first step in testing this hypothesis was to determine if glucose-fed SP cells produce the precursors for acetyl-CoA synthesis. Acid soluble intracellular metabolites were obtained from clarified cell lysates. Targeted metabolic profiling of these samples was performed by ^1H NMR spectroscopy (Saude et al., 2006). In this quantitative method, NMR spectra of purified

compounds at known concentrations are compared to spectra collected from cell samples. Representative spectra for glucose in one refeeding experiment are shown in Figure 5.4 B. As expected the concentration of glucose is very low in unfed cells, and rises sharply after refeeding (Figure 5.4 B and C; the upper panel includes the reference signature peaks for glucose; the lower panel shows glucose in cells). These changes occur in concert with glucose depletion from the medium (Figure 3.1 B). Increases in cellular ADP, ATP, acetate and ethanol concentrations accompany glucose uptake (Figure 5.4 C). An enzymatic assay confirmed acetate production in glucose-fed SP cells (Figure 5.4 D). We conclude that glucose feeding in SP results in new synthesis of ATP and acetate, the metabolites required for acetyl-CoA production.

The acetyl-CoA used by nuclear KATs is synthesized by acetyl-CoA synthase (Acs) 1 and/or 2 (Takahashi et al., 2006). If glucose induction of acetylation in SP depends on increased synthesis of acetyl-CoA for use by KATs, then Acs1 and/or Acs2 should be expressed in SP, and necessary for glucose induction of acetylation. Figure 5.5 A shows that Acs1 and Acs2 are both present in SP cells. Acs1 expression in SP is likely due, at least

in part, to derepression of *ACS1* transcription under conditions of glucose limitation (van den Berg et al., 1996). Note that although glucose refeeding might inhibit *ACS1* transcription in SP, *Acs1* protein expression remains high during the period when histones are acetylated (Figure 5.5 A), and enzyme activity declines only slowly under conditions that repress *ACS1* transcription (de Jong-Gubbels et al., 1997). Therefore acetyl-CoA synthesis in SP cells treated with glucose could result from the activity of both *Acs1* and *Acs2*.

We tested the dependence of acetylation on acetyl-CoA synthase (ACS) activity using strains that harbor a deletion allele of *ACS1*, a temperature sensitive allele of *ACS2* (*acs2-Ts1*), or both (Takahashi et al., 2006). Figure 5.5 B shows that H3 K9 and H4 acetylation decline in SP when *Acs1* is absent and *acs2-Ts1* is thermally inactivated; therefore maintenance of acetylation in SP requires acetyl-CoA synthesis (by either enzyme). At the permissive temperature, the *acs1Δ* and *acs2-Ts1* mutations in combination have no effect on maintenance acetylation, but glucose induction of H4ac is consistently dampened (Figure 5.5 B, lanes 15, 16, 7, 8). If *acs2-Ts1* protein is partly defective for acetyl-CoA production at room

temperature, then the blunted response in the double mutant might reflect the dependence of glucose induction of acetylation on increased metabolic flux through the ACS enzymes. It follows that the difference in induction of histone acetylation between mutant and wild type cells could be accentuated at lower temperatures, where reaction rates are slowed. Consistent with this prediction, at 16°C induction of H4ac by glucose is robust in wild type cells but is not evident in *acs1Δ acs2-Ts1* cells even at 2 h after refeeding (Figure 5.5 C; note that both strains are viable at 16°C). We conclude that ACS activity is essential for glucose induction of acetylation. From a broader perspective, we hypothesize that increased glycolysis provides the metabolites necessary for increased flux through the ACSs, which in turn expands an acetyl-CoA pool that is used by untargeted KATs for histone acetylation.

A strong prediction of this hypothesis is that induction of histone acetylation requires execution of all the steps of glycolysis. Consistent with this prediction, the glucose analogue 2-deoxy-D-Glucose (2DG), which is not metabolized after being phosphorylated in the first step of glycolysis (Figure 5.4 A), does not induce histone acetylation (Figure 5.6 A). Feeding a 1:1

mixture of glucose and 2DG dampens the response in comparison to feeding of glucose alone (Figure 5.6 A), probably because 2DG phosphorylation consumes ATP and blocks ATP production by glycolysis, thereby reducing ATP availability for acetyl-CoA synthesis.

To further test if glucose catabolism is important for glucose-stimulated histone acetylation, we analyzed glucose regulation of H4ac in a strain defective in pyruvate kinase 1 (Pyk1, encoded by *CDC19*), which catalyzes the final step of glycolysis (Figure 5.4 A, phosphoenolpyruvate → pyruvate). Note that Pyk1 inactivation does not deplete pre-existing ATP stores because ATP consumption during the hexose phase of glycolysis is balanced by its production in the subsequent steps leading to phosphoenolpyruvate synthesis (Figure 5.4 A). As shown in Figure 5.6 B (lanes 1, 3, 5, 7), unfed wild type and *PYK1* mutant cells (Pringle and Hartwell, 1981) have the same level of acetylation at the permissive and restrictive temperatures. The mutant, however, does not support induction of H4ac at either temperature (Figure 5.6 B, lanes 1, 2 compared to 3, 4; 5, 6 compared to 7, 8). We conclude that flux through Pyk1 allows for both net ATP generation by glycolysis and production of

pyruvate which is used to form acetate that is required for acetyl-CoA synthesis. Glucose induction of histone acetylation in SP is thus critically dependent on its metabolism to produce the acetyl-CoA used by untargeted KATs.

5.2.3 Examining the sensitivity of overall histone acetylation to energy deprivation

Our examination of glucose refeeding demonstrated that the global acetylation state seems to depend upon flux through the ACS enzymes to maintain a pool of acetyl CoA. When glucose is depleted in batch culture cells undergo a metabolic reprogramming in what is called the “diauxic shift”. This reprogramming allows cells to metabolize alternative carbon sources in the media including the ethanol that was generated by fermentative metabolism (Gray et al., 2004). Since the ACS enzymes require ATP and acetate for synthesis of acetyl-CoA, global acetylation levels should be sensitive to the energy state of the cell. Therefore, one would expect global acetylation levels to drop rapidly after glucose depletion if cells were unable to engage in respiratory metabolism following depletion of fermentable carbon sources. Several components of the respiratory chain are encoded by the mitochondrial DNA

(mtDNA). Loss of the mtDNA leaves cells viable but incapable of respiratory metabolism. Cells with mtDNA are referred to as “p⁺” and those that have lost the mtDNA as “p⁰”. Consistent with our expectation that induction of respiratory metabolism is important for normal regulation of histone acetylation, p⁰ cells displayed much lower levels of acetylated histone H4 than p⁺ cells did after 7 days growth in YPD (Figure 5.7 A). A similar effect was observed when respiration was disrupted by: 1) deletion of nuclear genes encoding components of the mitochondrial translation machinery, *PET112* and *PET123* (Figure 5.7 B), 2) deletion of components of the Hap2/3/4/5 complex which globally regulates transcription of respiratory genes, or 3) deletion of *SNF1*, the kinase which orchestrates signalling events required for derepression of respiratory genes after glucose depletion (Schuller, 2003) (Figure 5.7 C). Together, these results indicate that loss of acetylation in these mutants occurs as a result of loss of respiratory capacity rather than loss of some function specific to one of these genes.

The acetylation level was lower in p⁰ cells than in p⁺ cells, specifically at sites on H4 (K5, K8, and K12) where acetylation was increased after glucose refeed (Figure 3.2 C and Figure 4.7,

A). Levels of acetylation at H4 K16 were not inducible by glucose refeed (Figure 3.2 C), and were not lower in p^0 cells than in p^+ cells (Figure 5.7 A). This suggests that K16 is not subject to regulation by the mechanisms that tie overall acetylation to cellular energy state. This may be desirable given the established role of acetylation at K16 of H4 in regulation of chromatin condensation and maintenance of euchromatic regions of DNA (Shogren-Knaak et al., 2006).

Rheostat-like control of global histone acetylation could be exerted by glycolytic metabolism through control of energy balance. We analyzed this possibility by transferring WT p^+ cells from log phase cultures to water, or water containing various concentrations of glucose or 2-deoxy-D-glucose. Water provides an external environment devoid of metabolites, glucose can be used to generate ATP, and metabolism of 2-deoxy-D-glucose depletes ATP. Comparing cells incubated in water to those in glucose, we see that histone acetylation level increases with increasing amounts of glucose (Figure 5.8). Conversely histone acetylation levels decline with increasing concentrations of 2-deoxy-D-glucose (Figure 5.8). In this simplified system, overall acetylation increases with increased potential for energy

production and decreases with depletion of energy. This is what we would expect if glycolysis exerted rheostat like control of global histone acetylation.

5.3 Conclusions

The regulatory mechanism governing untargeted KAT activity appears to allow direct coupling of metabolic rate with global acetylation level. Under conditions of glucose deprivation, the rapid increase in glycolytic metabolism appears to drive increased histone acetylation simply by providing the substrates for histone acetylation. When SP cells are fed glucose, these effects seem to be largely independent from the major nutrient signalling pathways that control most of the cellular response to glucose. It is of course possible that classical signalling might still be able to fine tune this system during less extreme variations in nutrient availability. Nutrient signalling pathways can affect the enzymatic activities of various metabolic enzymes. PKA phosphorylation of two glycolytic enzymes, PFK2 and PYK1, impinges on their activity (Dihazi et al., 2003; Portela et al., 2002; Portela et al., 2006). Future research could elucidate the effects of these types of signalling events on overall acetylation. Collectively, our results suggest that the metabolic regulation of

overall changes in histone acetylation can couple cellular energy level and global histone acetylation.

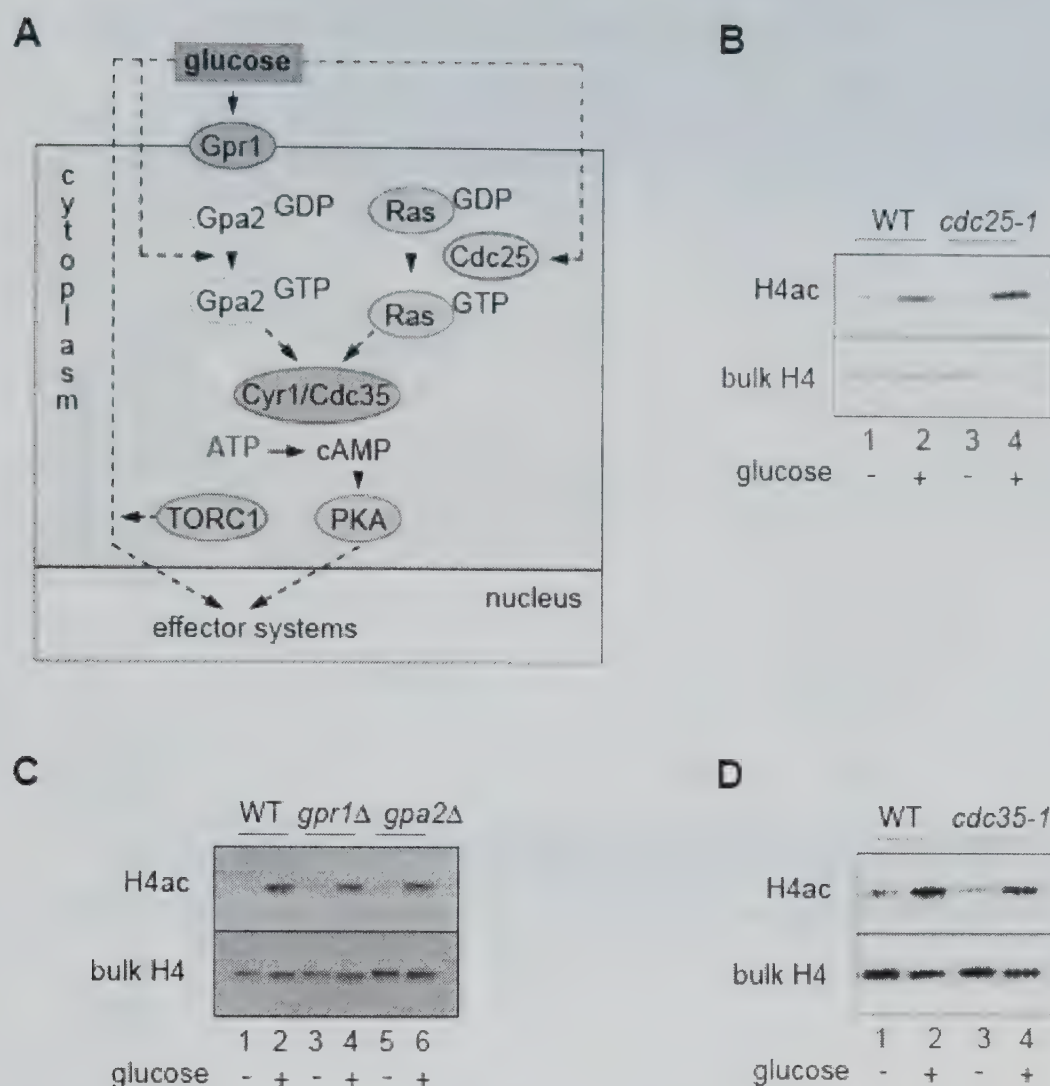


Figure 5.1 Neither Ras- nor Gpr1/Gpa2-dependent PKA signalling is 'necessary and sufficient' for glucose induction of histone acetylation in SP cells.

(A) Simplified outline of PKA- and TOR Complex 1-dependent glucose signalling in budding yeast (Zaman et al., 2008).

(B-D) Immunoblot analysis of H4 acetylation in the indicated strains at 1 h after glucose refeeding (control aliquots were untreated). In experiments with the temperature sensitive *cdc25-1* and *cdc35-1* strains (B and D respectively), wild type and mutant cells were shifted to 40°C for 30 min prior to refeeding, and cultured for 1 h before harvesting for protein isolation. Bulk H4 is the loading control.

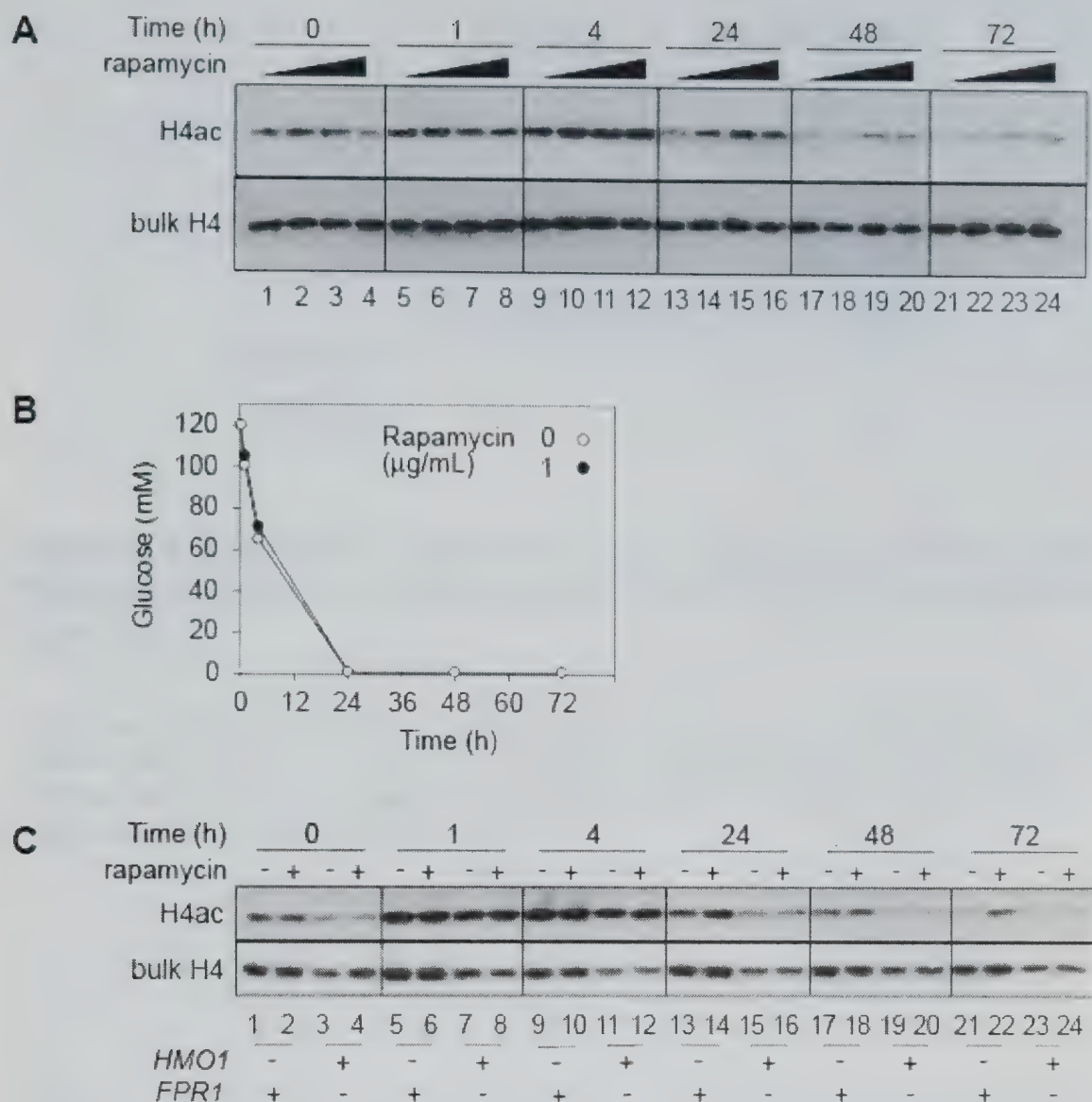


Figure 5.2 Effect of rapamycin treatment on the response of SP cells to glucose refeeding.

(A) Immunoblot analysis of the bulk expression of H4, and expression of acetylated H4, at the indicated time points after refeeding with 2% glucose in the presence of 0 – 1000 ng/mL rapamycin.

(B) Glucose concentration in the medium as a function of time after refeeding with glucose. Cells were treated with the drug vehicle or 1 μ g/mL rapamycin.

(C) Immunoblot analysis of the bulk expression of H4, and expression of acetylated H4, in *FPR1* and *fpr1* Δ strains. Cultures received 1 μ g/mL rapamycin or just the drug vehicle, and then glucose to a final concentration of 2%. The abnormal response of *fpr1* Δ cells to rapamycin does not depend on the strategy used to delete *FPR1* since the control strain contains another non-essential gene (*HMO1*) knocked out by the same approach.

The experiments in (A-C) were performed by B.Wu in consultation with me.

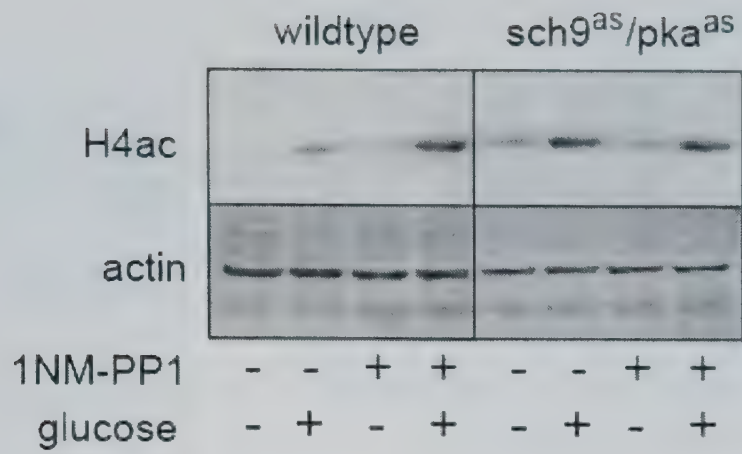


Figure 5.3 Neither separate nor combined inhibition of PKA and Sch9 can block overall acetylation response to glucose.

Eight day WT and Sch9^{as}PKA^{as} YPD +Ade cultures were split and treated with 100nM 1NM-PP1 or an equivalent volume of vehicle (DMSO). After 30min incubation cultures were adjusted to 2% glucose or left unfed. Cells were harvested 1hour after refeed.

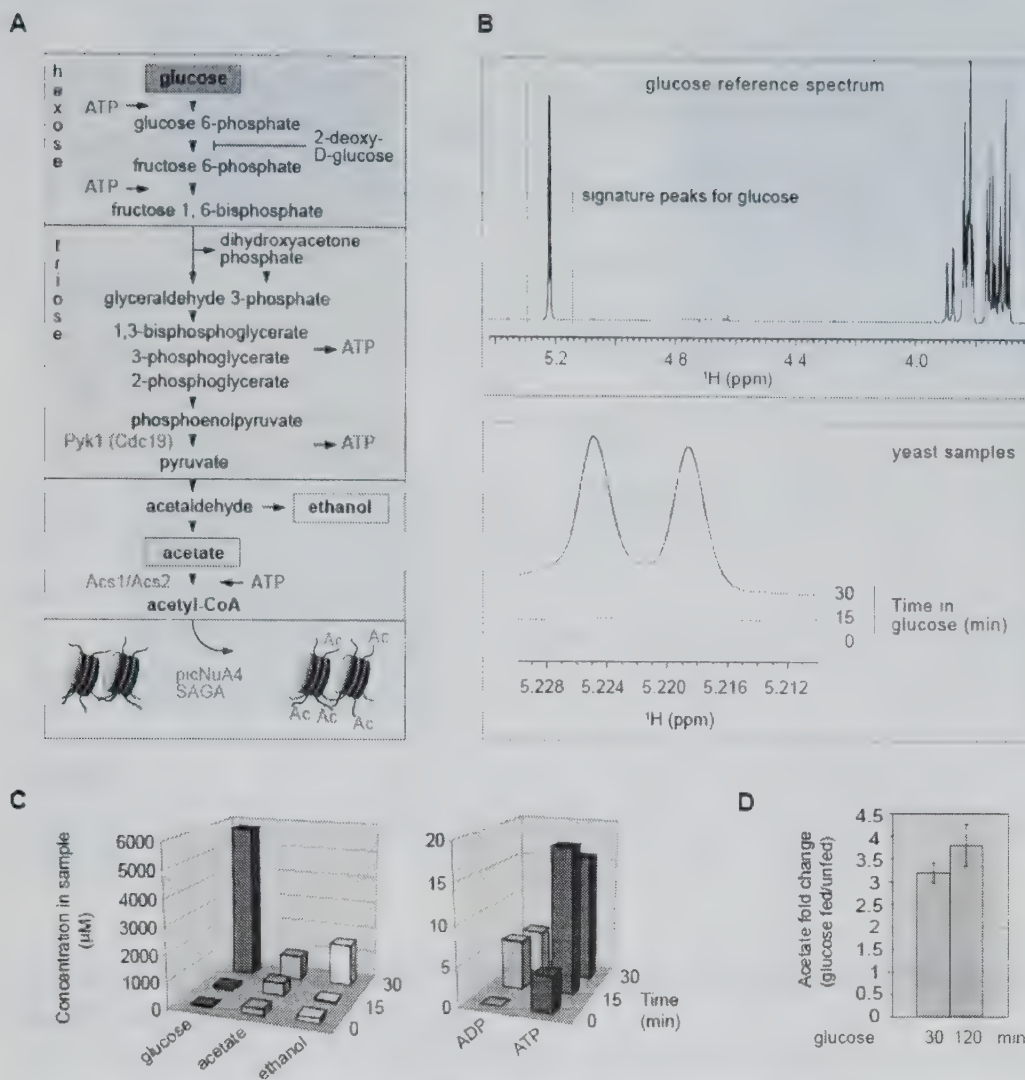


Figure 5.4 Metabolite profiles in unfed and glucose-fed SP cells.

(A) Relationship of glycolysis to acetyl-CoA production for use by nuclear KATs.

(B) ¹H-NMR spectra of a glucose reference sample (top panel) and samples of metabolites isolated from SP cells (bottom panel; only glucose peaks are shown).

(C) Targeted quantitative profiling of cellular metabolite levels by ¹H-NMR, before and after glucose refeeding. Identical volumes were analyzed from samples prepared from the same number of cells and resuspended in the same final volume. The results are the average of two independent experiments.

(D) Cellular level of acetate in glucose-fed cells relative to the level in unfed cells (average of 3 independent experiments ± S.D.). Acetate was measured by an enzymatic assay.

Spectra presented (B) and (C) were collected and interpreted by S. Reinke using samples from experiments which I conducted

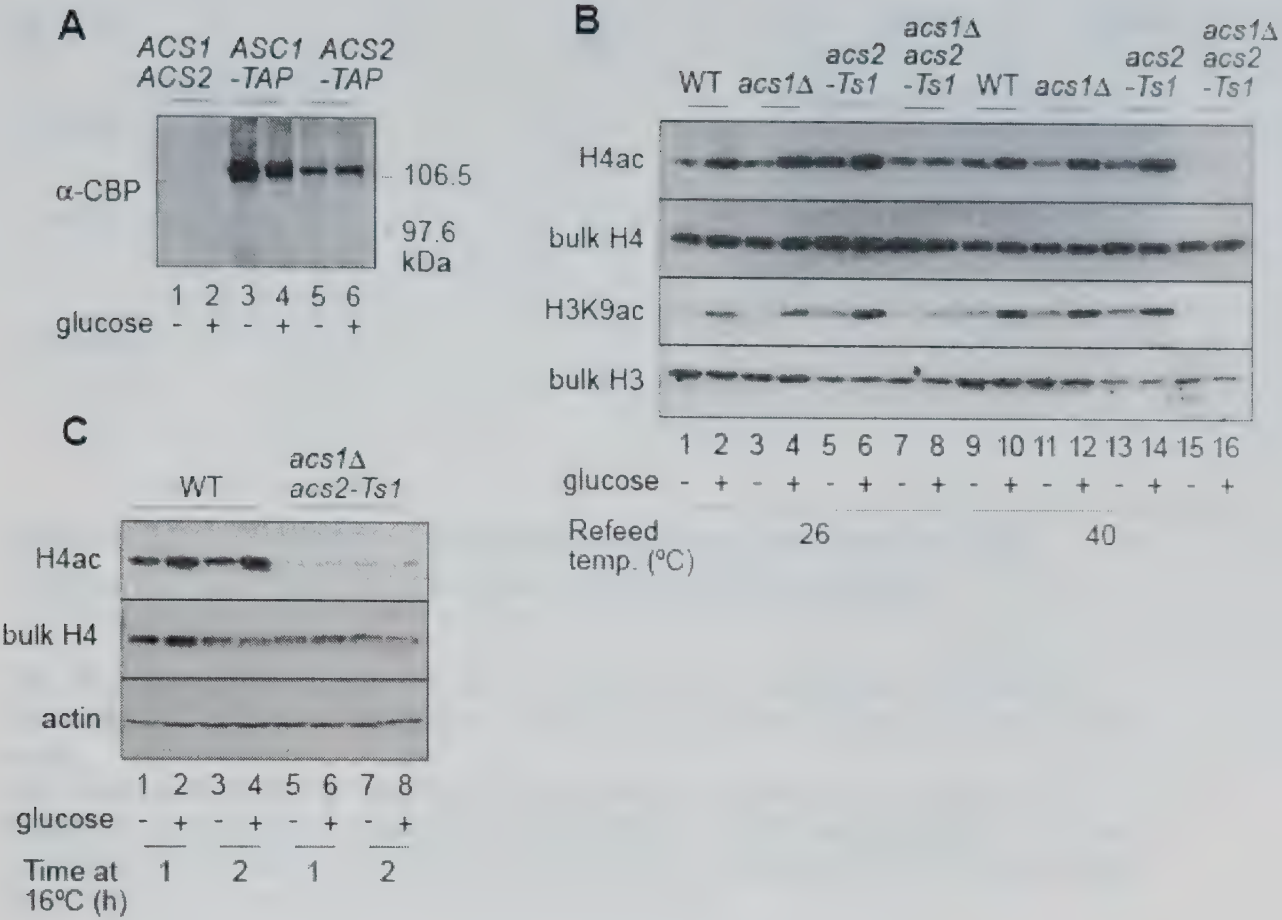


Figure 5.5 Metabolic regulation of global histone acetylation by glucose.

(A) Expression of TAP-tagged Acs1 and Acs2 in unfed and fed cells monitored by anti-CBP immunoblotting. Glucose-fed cells were harvested at 1 h.

(B) Immunoblotting analysis of H4 acetylation in wild type and *ACS1/ACS2* mutant strains. Cells were unfed or glucose-fed (1 h), at either the permissive (26°C) or restrictive (40°C) temperature.

(C) Immunoblotting analysis of H4 acetylation in wild type and *acs1Δ acs2-Ts1* mutant strains shifted from room temperature to 16°C at the time of glucose refeeding.

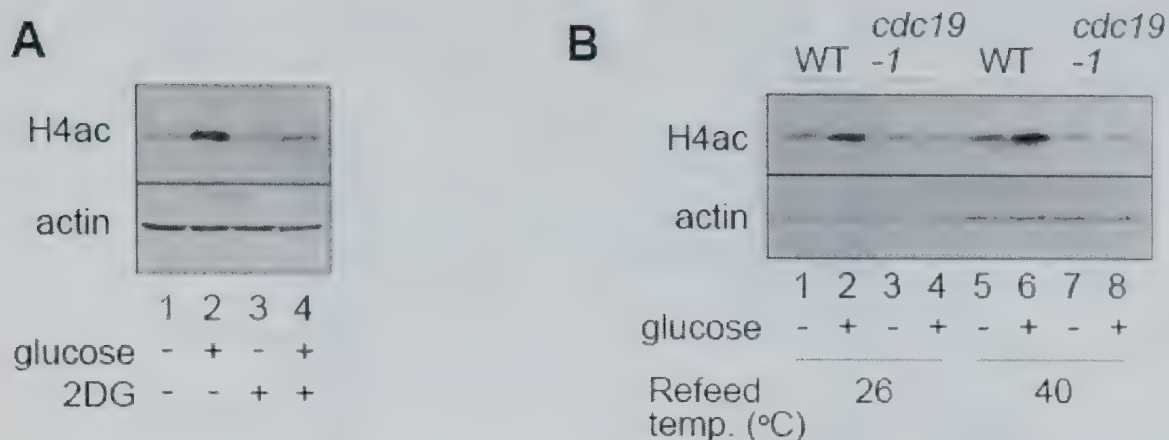


Figure 5.6 Glucose induction of acetylation in SP requires full glycolytic processing of glucose.

(A) Immunoblotting analysis of H4 acetylation in wild type cells fed 2% glucose, 2% 2-deoxy-D-glucose (2DG), or a mixture of both sugars at 1% each.

(B) Immunoblotting analysis of H4 acetylation in wild type and a *PYK1* (*CDC19*) temperature sensitive mutant strain. Cells were unfed or glucose-fed (1 h), at either the permissive (26°C) or restrictive (40°C) temperature.

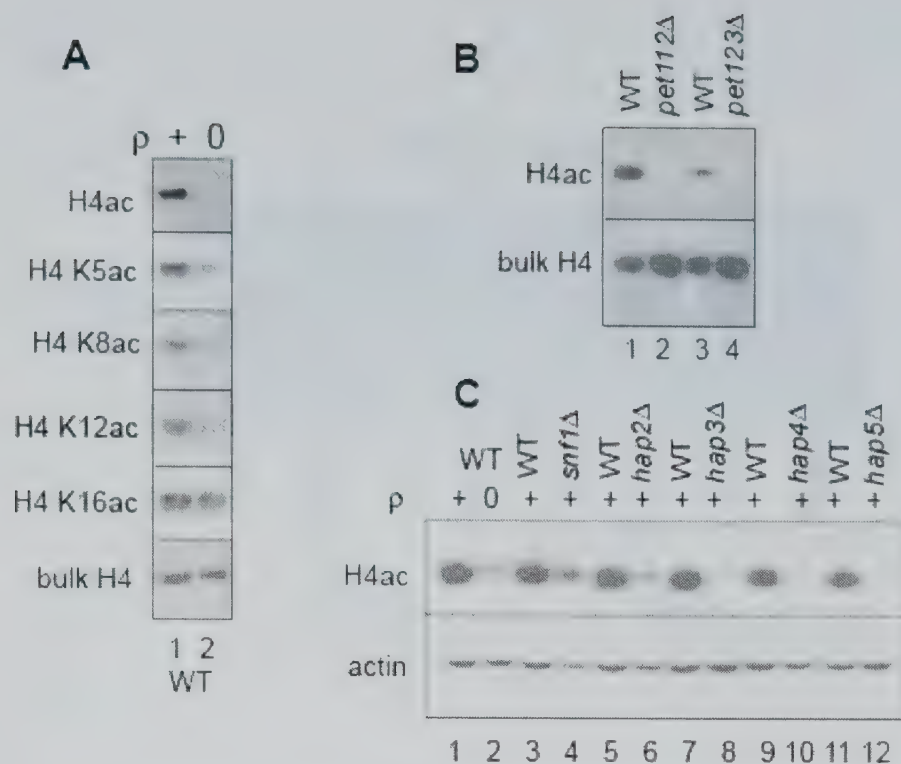


Figure 5.7 Respiratory deficient cells display very low levels of histone acetylation in SP.

(A) Cells with (ρ^+) or without (ρ^0) mitochondrial DNA were grown for 7 days in YPD at room temperature then harvested for use in Western analysis of histone H4 acetylation.

(B) Wild type cells and nuclear petite mutants *pet112 Δ* and *pet123 Δ* were grown for 7 days in YPD at room temperature then harvested for use in western analysis of histone H4 acetylation.

(C) Wild type, *snf1 Δ* , *hap2 Δ* , *hap3 Δ* , *hap4 Δ* , and *hap5 Δ* cells were grown for 7 days in YPD at room temperature then harvested for use in western analysis of histone H4 acetylation.

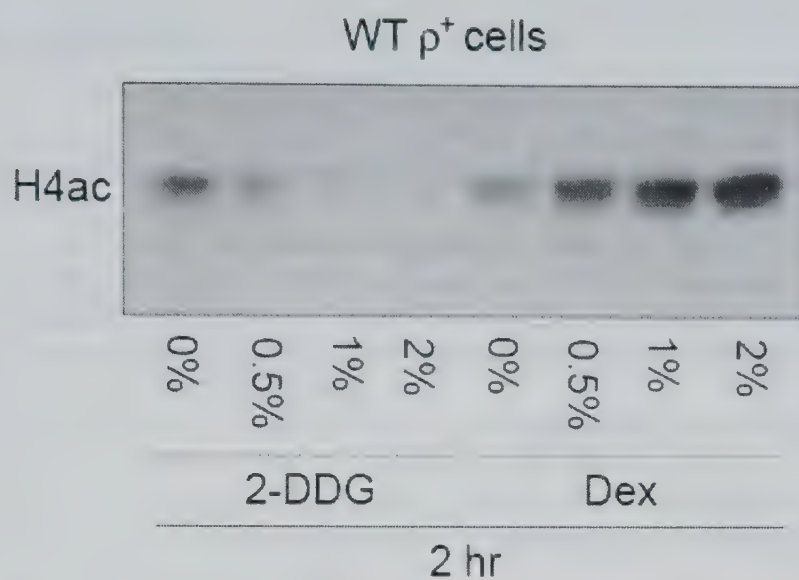


Figure 5.8 Overall acetylation on H4 decreases due to ATP depletion and increases with increased glucose supply.

Wild type ρ^+ cells were grown to early log then transferred to water containing either 0%, 0.5%, 1% or 2% glucose or 2-deoxy-d-glucose and harvested after 2 hours for analysis of histone H4 acetylation.

5.4 References

- Avvakumov, N. and Cote, J. (2007) The MYST family of histone acetyltransferases and their intimate links to cancer. *Oncogene*, **26**, 5395-5407.
- Barlev, N.A., Poltoratsky, V., Owen-Hughes, T., Ying, C., Liu, L., Workman, J.L. and Berger, S.L. (1998) Repression of GCN5 histone acetyltransferase activity via bromodomain-mediated binding and phosphorylation by the Ku-DNA-dependent protein kinase complex. *Mol Cell Biol*, **18**, 1349-1358.
- Chen, D. and Guarente, L. (2007) SIR2: a potential target for calorie restriction mimetics. *Trends Mol Med*, **13**, 64-71.
- Clarke, A.S., Lowell, J.E., Jacobson, S.J. and Pillus, L. (1999) Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol Cell Biol*, **19**, 2515-2526.
- de Jong-Gubbels, P., van den Berg, M.A., Steensma, H.Y., van Dijken, J.P. and Pronk, J.T. (1997) The *Saccharomyces cerevisiae* acetyl-coenzyme A synthetase encoded by the ACS1 gene, but not the ACS2-encoded enzyme, is subject to glucose catabolite inactivation. *FEMS Microbiol Lett*, **153**, 75-81.
- De Virgilio, C. and Loewith, R. (2006) Cell growth control: little eukaryotes make big contributions. *Oncogene*, **25**, 6392-6415.
- Dechant, R. and Peter, M. (2008) Nutrient signals driving cell growth. *Curr Opin Cell Biol*, **20**, 678-687.
- Dihazi, H., Kessler, R. and Eschrich, K. (2003) Glucose-induced stimulation of the Ras-cAMP pathway in yeast leads to multiple phosphorylations and activation of 6-phosphofructo-2-kinase. *Biochemistry*, **42**, 6275-6282.
- Durant, M. and Pugh, B.F. (2006) Genome-wide relationships between TAF1 and histone acetyltransferases in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **26**, 2791-2802.
- Gray, J.V., Petsko, G.A., Johnston, G.C., Ringe, D., Singer, R.A. and Werner-Washburne, M. (2004) "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev*, **68**, 187-206.
- Grubisha, O., Smith, B.C. and Denu, J.M. (2005) Small molecule regulation of Sir2 protein deacetylases. *Febs J*, **272**, 4607-4616.
- Heitman, J., Movva, N.R. and Hall, M.N. (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*, **253**, 905-909.
- Huang, W.C. and Chen, C.C. (2005) Akt phosphorylation of p300 at Ser-1834 is essential for its histone acetyltransferase and transcriptional activity. *Mol Cell Biol*, **25**, 6592-6602.
- Imai, S., Armstrong, C.M., Kaeberlein, M. and Guarente, L. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*, **403**, 795-800.
- Kresnowati, M.T., van Winden, W.A., Almering, M.J., ten Pierick, A., Ras, C., Knijnenburg, T.A., Daran-Lapujade, P., Pronk, J.T., Heijnen, J.J. and Daran, J.M. (2006) When transcriptome meets metabolome: fast cellular responses of yeast to sudden relief of glucose limitation. *Mol Syst Biol*, **2**, 49.

- Lafon, A., Chang, C.S., Scott, E.M., Jacobson, S.J. and Pillus, L. (2007) MYST opportunities for growth control: yeast genes illuminate human cancer gene functions. *Oncogene*, **26**, 5373-5384.
- Landry, J., Sutton, A., Tafrov, S.T., Heller, R.C., Stebbins, J., Pillus, L. and Sternglanz, R. (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci U S A*, **97**, 5807-5811.
- Lemaire, K., Van de Velde, S., Van Dijck, P. and Thevelein, J.M. (2004) Glucose and sucrose act as agonist and mannose as antagonist ligands of the G protein-coupled receptor Gpr1 in the yeast *Saccharomyces cerevisiae*. *Mol Cell*, **16**, 293-299.
- Lemercier, C., Legube, G., Caron, C., Louwagie, M., Garin, J., Trouche, D. and Khochbin, S. (2003) Tip60 acetyltransferase activity is controlled by phosphorylation. *J Biol Chem*, **278**, 4713-4718.
- Lottersberger, F., Panza, A., Lucchini, G. and Longhese, M.P. (2007) Functional and physical interactions between yeast 14-3-3 proteins, acetyltransferases, and deacetylases in response to DNA replication perturbations. *Mol Cell Biol*, **27**, 3266-3281.
- McGee, S.L., van Denderen, B.J., Howlett, K.F., Mollica, J., Schertzer, J.D., Kemp, B.E. and Hargreaves, M. (2008) AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes*, **57**, 860-867.
- Millar, C.B. and Grunstein, M. (2006) Genome-wide patterns of histone modifications in yeast. *Nat Rev Mol Cell Biol*, **7**, 657-666.
- Nagy, Z. and Tora, L. (2007) Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene*, **26**, 5341-5357.
- Portela, P., Howell, S., Moreno, S. and Rossi, S. (2002) In vivo and in vitro phosphorylation of two isoforms of yeast pyruvate kinase by protein kinase A. *J Biol Chem*, **277**, 30477-30487.
- Portela, P., Moreno, S. and Rossi, S. (2006) Characterization of yeast pyruvate kinase 1 as a protein kinase A substrate, and specificity of the phosphorylation site sequence in the whole protein. *Biochem J*, **396**, 117-126.
- Pringle, J.R. and Hartwell, L.H. (1981) The *Saccharomyces cerevisiae* cell cycle. pp. 97-142 in: *The molecular biology of the yeast Saccharomyces. Life cycle and inheritance. 1981* Edited by Strathern, J.N., Jones, E.W. and Broach, J.R. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA.
- Saude, E.J., Slupsky, C.M. and Sykes, B.D. (2006) Optimization of NMR analysis of biological fluids for quantitative accuracy. *Metabolomics*, **2**, 113-123.
- Schuller, H.J. (2003) Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. *Curr Genet*, **43**, 139-160.
- Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R. and Peterson, C.L. (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science*, **311**, 844-847.
- Smith, E.R., Eisen, A., Gu, W., Sattah, M., Pannuti, A., Zhou, J., Cook, R.G., Lucchesi, J.C. and Allis, C.D. (1998) ESA1 is a histone acetyltransferase that is essential for growth in yeast. *Proc Natl Acad Sci U S A*, **95**, 3561-3565.

- Smith, J.S., Brachmann, C.B., Celic, I., Kenna, M.A., Muhammad, S., Starai, V.J., Avalos, J.L., Escalante-Semerena, J.C., Grubmeyer, C., Wolberger, C. and Boeke, J.D. (2000) A phylogenetically conserved NAD⁺-dependent protein deacetylase activity in the Sir2 protein family. *Proc Natl Acad Sci U S A*, **97**, 6658-6663.
- Takahashi, H., McCaffery, J.M., Irizarry, R.A. and Boeke, J.D. (2006) Nucleocytoplasmic acetyl-coenzyme A synthetase is required for histone acetylation and global transcription. *Mol Cell*, **23**, 207-217.
- Thaminy, S., Newcomb, B., Kim, J., Gatbonton, T., Foss, E., Simon, J. and Bedalov, A. (2007) Hst3 is regulated by Mec1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. *J Biol Chem*, **282**, 37805-37814.
- Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J.D., McMullen, B., Hurwitz, M., Krebs, E.G. and Wigler, M. (1987) Cloning and characterization of BCY1, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **7**, 1371-1377.
- Urban, J., Soulard, A., Huber, A., Lippman, S., Mukhopadhyay, D., Deloche, O., Wanke, V., Anrather, D., Ammerer, G., Riezman, H., Broach, J.R., De Virgilio, C., Hall, M.N. and Loewith, R. (2007) Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol Cell*, **26**, 663-674.
- van den Berg, M.A., de Jong-Gubbels, P., Kortland, C.J., van Dijken, J.P., Pronk, J.T. and Steensma, H.Y. (1996) The two acetyl-coenzyme A synthetases of *Saccharomyces cerevisiae* differ with respect to kinetic properties and transcriptional regulation. *J Biol Chem*, **271**, 28953-28959.
- Wang, Y., Pierce, M., Schnepfer, L., Guldal, C.G., Zhang, X., Tavazoie, S. and Broach, J.R. (2004) Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. *PLoS Biol*, **2**, E128.
- Yuan, L.W., Soh, J.W. and Weinstein, I.B. (2002) Inhibition of histone acetyltransferase function of p300 by PKCdelta. *Biochim Biophys Acta*, **1592**, 205-211.
- Zaman, S., Lippman, S.I., Schnepfer, L., Slonim, N. and Broach, J.R. (2009) Glucose regulates transcription in yeast through a network of signaling pathways. *Mol Syst Biol*, **5**, 245.
- Zaman, S., Lippman, S.I., Zhao, X. and Broach, J.R. (2008) How *Saccharomyces* Responds to Nutrients. *Annu Rev Genet*.

Chapter 6: Mitochondrial defects impair the transcriptional response to removal of glucose

6.1 Introduction

Regulation of growth and maintenance of homeostasis requires that cells establish an appropriate response to the abundance and type of nutrients available in their environment. The optimal response to glucose in *Saccharomyces cerevisiae* is achieved through regulation of the metabolic and transcriptional apparatus by protein kinase A (PKA) and the protein kinase Snf1. In the presence of glucose PKA is active and Snf1 is inactive (Jiang and Carlson, 1996; McCartney and Schmidt, 2001; Wilson et al., 1996). When glucose is depleted, Snf1 becomes active and PKA activity drops, allowing cells to undergo a transcriptional and metabolic reprogramming known as the diauxic shift. The diauxic shift allows metabolism of other carbon sources in the media including the ethanol that was produced by fermentation. In this way the diauxic shift facilitates continued survival.

6.1.1 Glucose-dependent signal transduction

The catalytic subunit of yeast PKA is encoded redundantly by three genes, *TPK1*, *TPK2*, and *TPK3* (Toda et al., 1987b). These catalytic subunits associate as a dimer which is bound to

and inactivated by a dimer of regulatory subunits encoded by *BCY1* (Toda et al., 1987a). Dissociation of Bcy1 from the catalytic PKA subunits is triggered by binding of second messenger cAMP to Bcy1.

Glucose induces generation of cAMP through two pathways. Both pathways promote cAMP synthesis through activation of the adenylate synthase Cyr1 by a small GTP binding protein (Broek et al., 1985; Peeters et al., 2006; Toda et al., 1985). Through an uncharacterized mechanism, intracellular metabolism of glucose results in activation of the GTP binding Ras proteins, Ras1 and Ras2. This regulation is absolutely required for glucose induction of cAMP. Glucose does not trigger increases in cAMP in *ras1 ras2* double mutants (Mbonyi et al., 1988).

Extracellular glucose can exert effects on cAMP production through activities of the G protein coupled receptor homologue Gpr1, and the small GTP binding protein Gpa2 (Kraakman et al., 1999; Lemaire et al., 2004; Xue et al., 1998). Gpa2 activation via GTP loading is triggered by Gpr1 interaction with its ligands glucose or sucrose (Lemaire et al., 2004). Activation of this pathway encourages cAMP synthesis but is not absolutely required (Rolland et al., 2000). The Ras proteins likely mediate

initial activation of adenylate cyclase, and the Gpr1 Gpa2 pathway modulates cyclase activity during continued growth.

The activity of the Snf1 kinase is regulated by autoinhibition, subcellular localization, and phosphorylation (Celenza and Carlson, 1989; Hedbacker et al., 2004a; Hedbacker et al., 2004b; Jiang and Carlson, 1996; McCartney and Schmidt, 2001; Vincent et al., 2001). Like its mammalian counterpart, the AMP sensitive kinase AMPK, the Snf1 kinase is a heterotrimer consisting of a catalytic α subunit, a β subunit, and a γ subunit (Celenza and Carlson, 1989; Celenza et al., 1989; Erickson and Johnston, 1993; Yang et al., 1992; Yang et al., 1994). This heterotrimer remains intact in the presence or absence of glucose. *SNF1* encodes the catalytic subunit which contains an N-terminal kinase domain and a C-terminal regulatory domain (RD). Interaction between these domains in the presence of glucose keeps the kinase inactive (Jiang and Carlson, 1996). In the absence of glucose this autoinhibition is relieved through interaction of the γ subunit, Snf4, with the RD (Jiang and Carlson, 1996). Snf4 is essential for Snf1 activity (Celenza and Carlson, 1989; Celenza et al., 1989).

The three β subunits of the Snf1 kinase are encoded by the *S. cerevisiae* genes *GAL83*, *SIP1* and *SIP2* (Erickson and Johnston, 1993; Yang et al., 1992; Yang et al., 1994). The C-terminal domain of β subunits bridge the α and γ subunits, thereby providing a scaffold for the Snf1 complex. The divergent N-terminal regions of the three β subunits appear to play regulatory roles in directing subcellular localization of the complex. During growth on glucose all three β subunits are cytoplasmic. Exhaustion or removal of extracellular glucose triggers relocation of Gal83 complexes to the nucleus and Sip1 complexes to the vacuole, while Sip2 complexes remain in the cytoplasm (Vincent et al., 2001).

Finally, phosphorylation at Thr210 in the Snf1 activation loop is required for activation of the kinase (McCartney and Schmidt, 2001). Phosphorylation of Thr210 is regulated by three upstream kinases Sak1, Elm1, and Tos3 and by the Reg1-Glc7 phosphatase (Hong et al., 2003; Nath et al., 2003; Sanz et al., 2000; Sutherland et al., 2003; Tu and Carlson, 1995). This is analogous to the phosphorylation of Thr172 in mammalian AMPK (Hawley et al., 1996) LKB1, CaMKK α and CaMKK β , the mammalian kinases which bear the most similarity in their

kinase domains to the yeast kinases that phosphorylate Snf1, carry out phosphorylation of AMPK Thr172 (Hawley et al., 2003; Hawley et al., 2005; Hong et al., 2003; Hurley et al., 2005; Shaw et al., 2004; Woods et al., 2005; Woods et al., 2003). The transforming growth factor- β (TGF β)-activated kinase-1 (TAK1) may also phosphorylate AMPK, as TAK1 is capable of activating Snf1 when expressed in yeast (Momcilovic et al., 2006). Protein phosphatase-2C is believed to dephosphorylate Thr172 (Sanders et al., 2007). Unlike AMPK, Snf1 does not appear to be allosterically activated by AMP. Activation does occur under circumstances when the cellular AMP:ATP ratio increases (Wilson et al., 1996) and glucose clearly antagonizes Snf1 activation.

6.1.2 Glucose regulation of transcription

Active PKA promotes anabolic cellular metabolism through induction of ribosome biogenesis, and protein synthesis (Zaman et al., 2008). Additionally PKA activity represses transcription of genes involved in the general stress response, nutrient metabolism, and meiosis. The Msn2 and Msn4 Cys₂ His₂ zinc-finger transcription factors activate transcription of genes with stress responsive elements (STRE) in their promoters (Gorner et

al., 1998). PKA phosphorylation of the Msn2 nuclear localization signal (NLS) prevents nuclear accumulation of Msn2 (Gorner et al., 1998). Therefore Msn2 and Msn4 are localized in the cytoplasm when PKA activity is high and in the nucleus when PKA activity is low or absent. Msn2 and Msn4 seem to regulate transcription of genes which suppress cellular growth. Similar to starvation for nutrients, inhibition of PKA leads to cell cycle arrest in G1 (Matsumoto et al., 1982). Inhibiting Msn2 Msn4 activity is an essential function for PKA in directing growth, as deletion of *MSN2* and *MSN4* allows growth of *tpk1Δ/tpk2Δ/tpk3Δ* cells, which are otherwise inviable (Smith et al., 1998).

PKA activity also facilitates transcriptional repression by Sok2 (Enjalbert et al., 2004; Shenhar and Kassir, 2001; Ward et al., 1995). Sok2 seem to repress genes which are inhibitory to growth. Overexpression of *SOK2* can also allow growth of *tpk1Δ/tpk2-ts/tpk3Δ* cells at restrictive temperature (Ward and Garrett, 1994). Additionally, PKA prevents transcriptional activation of a number of genes, regulated by Ume6, that are involved in nutrient metabolism and meiosis (Malathi et al., 1997; Rubin-Bejerano et al., 1996; Williams et al., 2002). Ume6, a stable component of the RPD3L HDAC complex, is a

transcription factor that binds URS1_A (5'-TCGGCGGCT-3') and URS1_B (5'-SGWGGMRRNANW-3') elements in gene promoters (Williams et al., 2002). Recruitment of RPD3L to these promoters results in localized deacetylation of histones and transcriptional repression (Carrozza et al., 2005). Transcriptional repression at these genes can be relieved by phosphorylation of Ume6, which allows Ume6 to act as a transcriptional activator (Malathi et al., 1997; Xiao and Mitchell, 2000). This phosphorylation is mediated by Rim15 and the glycogen synthase kinase 3 β (GSK3 β) homologue Rim11, both of which are phosphorylated and inhibited by PKA (Rubin-Bejerano et al., 1996; Swinnen et al., 2006; Xiao and Mitchell, 2000). The Ras and Gpa2 mediated stimulation of cAMP synthesis declines with depletion of glucose and genes that had been repressed due to events mediated by PKA are activated.

Upon exhaustion of glucose, greater than 400 genes in metabolism of alternative carbon sources, respiration, nutrient transport, gluconeogenesis and meiosis undergo transcriptional induction which relies on the Snf1 Kinase (Young et al., 2003). These effects are mediated through two main mechanisms involving three transcriptional activators, Cat8, Sip4, Adr1 and

the transcriptional repressor Mig1. Snf1 mediates this transcriptional program through phosphorylation of these transcriptional activators and repressors.

Cat8 and Sip4, transcriptional activators that bind to carbon source responsive elements (CSRE consensus sequence YCCRTTNRNCCG), are activated by Snf1-dependent phosphorylation (Rahner et al., 1999; Vincent and Carlson, 1998). Additionally, transcriptional activation by Adr1, which was originally identified as a factor involved in transcriptional induction of the glucose-repressable alcohol dehydrogenase *ADH2*, requires Snf1 activity (Ciriacy, 1979; Denis, 1987). Mig1 binds to a GC rich consensus sequence found in the promoters of many glucose repressed genes. Repression of these genes is mediated by the recruitment of the Ssn6(Cyc8)-Tup1 corepressor by Mig1 (Treitel and Carlson, 1995; Tzamarias and Struhl, 1995). Snf1 phosphorylates Mig1 at four sites when glucose is absent (Ostling and Ronne, 1998; Smith et al., 1999; Treitel et al., 1998). This phosphorylation disrupts Mig1 interaction with Ssn6-Tup1 and relieves repression (DeVit and Johnston, 1999; Papamichos-Chronakis et al., 2000). Together with the effects of deletion of PKA, Snf1 mediated processes

allow maintenance of cellular homeostasis in the absence of glucose.

Activities that control acetylation of histones make a major contribution to transcriptional regulation. Targeting of enzymes that acetylate histones (KATs) to promoters aids transcription, whereas targeting of HDACs can repress it (Shahbazian and Grunstein, 2007). Both KATs and HDACs also work in an untargeted fashion, acetylating and deacetylating histones in both intergenic regions and open reading frames (ORFs), thereby establishing a “global” acetylation level (Vogelauer et al., 2000). Global acetylation itself affects transcription. The strength of interactions needed to initiate transcription, and the kinetics of transcriptional induction and repression are both determined by global acetylation level (Imoberdorf et al., 2006; Katan-Khaykovich and Struhl, 2002; Vogelauer et al., 2000). The combination of targeted and untargeted acetylation constitutes the overall histone acetylation state. Unexpectedly, we discovered a link between mitochondrial function and Snf1- and PKA-mediated signaling while investigating of the effects of overall histone acetylation on the transcriptional response to glucose depletion.

6.2 Results and discussion

6.2.1 ρ^0 cells lose overall acetylation of H4 in response to glucose removal

We have discovered that cells lacking mitochondrial DNA (mtDNA), ρ^0 cells, and cells with mtDNA, ρ^+ cells, display similar overall histone acetylation levels during proliferative growth (Figure 6.1 lanes 1 and 2). Our previous analysis had shown that ρ^0 cells display greater reductions in overall histone acetylation than ρ^+ cells as they enter stationary phase (SP) (Figure 5.7 A). We speculated that decline in overall histone acetylation in SP might be specifically due to depletion of glucose. Overall histone acetylation levels are likely maintained in ρ^+ cells by use of respiratory carbon sources and intra-cellular carbohydrate stores. In contrast, ρ^0 cells are unable to engage in respiratory metabolism due to loss of components of the respiratory chain encoded in the mtDNA, and are known to have defects in the accumulation and mobilization of storage carbohydrates (Enjalbert et al., 2000; Yang et al., 1998). We hypothesized that, due to these metabolic defects, the overall histone acetylation level in ρ^0 cells is likely to be more sensitive to

removal of glucose than overall histone acetylation level of p^+ cells.

To test this hypothesis, p^+ and p^0 cells were grown to log phase in a medium called Synthetic Dextrose Complete (SDC), which provides only amino acids, ammonium, and glucose. Cells from these cultures were washed in the same medium without glucose (S_C) then transferred into S_C. After 4 hours continued incubation in S_C, p^+ cells display only minor, if any, decline in overall histone acetylation in comparison to log levels (Figure 6.1 compare lanes 1 and 3). A major decline in overall histone acetylation was evident in p^0 cells after 4 hours in S_C in comparison log levels (Figure 5.1 compare lanes 2 and 4). Whether directly due to differences in metabolic status, or through some other mechanism, when glucose is depleted or removed from the growth medium, overall histone acetylation levels drop precipitously in p^0 cells but not p^+ cells.

6.2.2 Microarray analysis of p^0 cells reveals a defect in derepression of glucose repressed genes after glucose removal

Given the established ties between histone acetylation and transcriptional regulation, we wondered if transcriptional

abnormalities accompanied the marked drop in overall histone acetylation seen in ρ^0 cells. The effect on the transcriptional program in ρ^0 cells of transfer to media without glucose was analyzed in microarray experiments. SDC cultures of ρ^+ and ρ^0 cells were grown to 1 OD₆₀₀, washed once in S_C, resuspended at 1 OD₆₀₀ in S_C and incubated for 4hrs. RNA samples were taken from ρ^+ and ρ^0 cells from SDC 1 OD₆₀₀ cultures and again after 4 hours in S_C. This allowed mRNA expression of ρ^+ or ρ^0 cells in SDC to be compared to mRNA expression of the same cell type in S_C, and mRNA expression in ρ^+ in SDC or S_C could be compared to mRNA expression of ρ^0 cells in the same media.

Transcriptional analysis was carried out using the *S. cerevisiae* GeneChip YG_S98 (Affymetrix). Genes were grouped into eight groups: those that displayed greater expression in SDC than in S_C (ρ^+ log > ρ^+ S_C and ρ^0 log > ρ^0 S_C), those that showed higher expression in S_C than SDC (ρ^+ S_C > ρ^+ log, and ρ^0 S_C > ρ^0 log), those that displayed higher expression in ρ^+ cells than in ρ^0 cells (ρ^+ log > ρ^0 log and ρ^+ S_C > ρ^0 S_C), and those that displayed greater expression in ρ^0 cells than in ρ^+ cells (ρ^0 log > ρ^+ log and ρ^0 S_C > ρ^+ S_C). GeneSpring software

(Agilent Technologies) was used to group genes by gene ontology (GO) terms (Ashburner et al., 2000). The GO terms displaying the most significant enrichment for each of the eight comparisons are listed in table 6.1. This approach seems robust, since our comparison of transcription in log phase ρ^+ and ρ^0 cells agrees with previous studies. Specifically, as seen in earlier studies, there was overrepresentation of genes involved in “response to drug” (Table 6.1 $p = 3.37 \times 10^{-4}$) but not “mitochondrial signaling pathway” (synonymous with “retrograde response”). The retrograde response is repressed by glucose and induced only when ρ^0 cells are grown in a non-repressing carbon source such as raffinose (Epstein et al., 2001; Liu and Butow, 2006; Traven et al., 2001).

Based on the current understanding of the role of histone acetylation in transcriptional regulation, a logical hypothesis is that the lower level of overall histone acetylation in ρ^0 cells would reduce the transcriptional response of genes normally upregulated upon glucose removal. Studies by Imoberdorf *et al.* suggested that at lower global acetylation levels, stronger transcriptional activators are required to induce transcription (Imoberdorf et al., 2006). We were interested to see if specific

genes, or gene classes, regulated by certain transcription factors, displayed different sensitivities to loss of histone acetylation. To identify genes and gene classes that showed a dampened transcriptional response to glucose removal in ρ^0 cells in comparison to ρ^+ cells, we compared the list of genes upregulated in ρ^+ cells after removal of glucose (ρ^+ S_C > ρ^+ log in SDC) to genes which are upregulated in ρ^+ cells in comparison to ρ^0 cells after removal of glucose (ρ^+ S_C > ρ^0 S_C). Notably in both of these lists, we see significant enrichment of genes involved in “generation of precursor metabolites and energy”, “energy derivation by oxidation of organic compounds”, “cellular respiration”, and “aerobic respiration” (Table 6.1). However ρ^0 cells are not transcriptionally inactive, analysis suggests that ρ^0 cells upregulate ~240 genes more than two fold after transfer to S_C. Among these genes, those involved in monosaccharide transport are particularly enriched (Table 6.1 $p=4.39 \times 10^{-4}$).

Due to the rapid loss of acetylation in ρ^0 cells after glucose removal, we had expected to see dampening of the transcriptional response to glucose removal. In accordance with these expectations, some of the genes that are strongly upregulated after removal of glucose in ρ^+ cells such as *SUC2*

(13.3 fold upregulated) and *JEN1* (19.35 fold upregulated) are also upregulated in ρ^0 cells albeit to a lesser extent (*SUC2*, 2.5 and *JEN1* 2.2 fold upregulated).

6.2.3 Examination of Snf1 signaling in ρ^0 cells: clues from analysis of transcriptional reprogramming

The striking loss of overall histone acetylation displayed by ρ^0 cells could be partially responsible for the altered transcriptional response of ρ^0 cells to glucose removal. We realized however, that the alterations in gene expression after glucose removal could be the result of altered activity of signaling pathways. Many of the genes that are upregulated in ρ^+ cells in response to transfer to S_C are known to be repressed by the presence of glucose in the growth media. Transcription of these genes follows activation of the Snf1 kinase and deactivation of PKA (Schuller, 2003; Zaman et al., 2008). Therefore we examined regulation of both of these signaling pathways during entry into SP and after glucose removal.

As noted above in comparison to the response of ρ^+ cells, *SUC2* and *JEN1* transcriptional induction after glucose removal is strongly dampened in ρ^0 cells. These genes are prototypical

Snf1 regulated genes (Schuller, 2003). Snf1 exerts control over transcription through inhibition of the transcriptional repressor Mig1 and potentiation of the activities of the transcription factors Cat8, Sip4, and Adr1 (Ciriacy, 1979; Denis, 1987; Ostling and Ronne, 1998; Rahner et al., 1999; Smith et al., 1999; Treitel et al., 1998; Vincent and Carlson, 1998). In our data, substantial links are evident between this regulon, and the genes which display greater induction of transcription in ρ^+ cells than ρ^0 cells after glucose removal. These are genes that 1) display at least 2 fold greater expression in ρ^+ cells after transfer to S_C than during log growth on SDC 2) display at least 2 fold greater expression in S_C in ρ^+ cells than ρ^0 cells and 3) were not upregulated in ρ^+ cells in comparison to ρ^0 cells during growth on SDC.

Sequence analysis tools provided by yeastract.com were used to determine the percentage of the genes in this list for which regulation by Snf1-directed factors is documented (Monteiro et al., 2008; Teixeira et al., 2006). In this list, 23.1% of genes are regulated by Adr1, 16.8% by Abf1, 16.4% by Swi4, 13.3% by Mig1, and 12.6% by Cat8. Overrepresentation of sequences resembling the consensus binding sites for Abf1

($p=2.08759 \times 10^{-6}$) and Mig1 ($p=1.755 \times 10^{-7}$) was also detected using MUSA (**M**otif finding using an **UnS**upervised **A**pproach) (Monteiro et al., 2008; Teixeira et al., 2006).

6.2.4 Biochemical analysis of activation of Snf1 signaling

To determine if ρ^0 cells display a defect in activity of the Snf1 signaling pathway, we examined activation of the Snf1 kinase by phosphorylation, and followed phosphorylation of the Snf1 substrate Mig1 at different times after removal of glucose. Phosphorylation of Thr210 of Snf1 is a critical event in activation of the kinase (McCartney and Schmidt, 2001). We assessed the activation of Snf1 using an antibody directed against AMPK phosphorylated at Thr172, which has previously been used successfully to analyze Thr210 phosphorylation of Snf1 (Orlova et al., 2006; Sutherland et al., 2003). Bulk levels of Snf1 remain relatively stable in ρ^+ cells and ρ^0 cells throughout this experiment (Figure 6.2 A). No bands are detected by either the bulk Snf1 or phospho-Snf1 antibodies in *snf1 Δ* cells at any time point in the experiment (Figure 6.2 A lanes 3, 6, 9, 12, 15). In accordance with known regulation of Snf1, phosphorylation of Snf1 is undetectable in log phase cells (Figure 6.2 A lanes 1-3).

Snf1 is phosphorylated in both ρ^+ and ρ^0 cells by 15 minutes after glucose removal and remains phosphorylated throughout a 120 minute time course (Figure 6.2 A lanes 4,5,7,8,10,11,13,14). This is surprising given the reduced transcription of Snf1-regulated genes in ρ^0 cells.

Alterations in Snf1 localization also affect its activity. Upon glucose removal, Snf1 in complex with the Gal83 β subunit translocate from the cytoplasm to the nucleus where Snf1 phosphorylates Mig1 at four sites, thereby relieving Mig1 mediated transcriptional repression (DeVit and Johnston, 1999; Ostling and Ronne, 1998; Papamichos-Chronakis et al., 2000; Smith et al., 1999; Treitel et al., 1998; Vincent et al., 2001). When phosphorylated by Snf1, Mig1 displays a notable mobility shift when resolved by SDS-PAGE (Ostling and Ronne, 1998; Treitel et al., 1998). We followed mobility shifts displayed by Mig1 after glucose removal using a strain expressing a TAP tagged form of Mig1. A shift to lower mobility forms of Mig1 is evident in both ρ^+ and ρ^0 cells by 15 minutes after glucose removal and remains so throughout a 120 minute time course (Figure 6.2 B compare lanes 1 and 2 to lanes 3-10). While Mig1 displays a mobility shift in both ρ^+ and ρ^0 cells, it is evident that

the shift displayed by ρ^0 cells is less than that seen in ρ^+ cells (Figure 6.2 B). Overall, Snf1 appears to be activated normally in ρ^0 cells and is capable of phosphorylating key substrates in the nucleus, albeit with a somewhat diminished alacrity.

6.2.5 Analysis of the PKA signaling pathway

The small differences in the phosphorylation of Mig1 (Figure 6.2 B) seem unlikely to be responsible for the transcriptional disturbance seen in ρ^0 cells. To help elucidate what signaling defects might be responsible for these transcriptional defects, we therefore examined other factors involved in the transcriptional response to glucose. PKA activity during growth on glucose represses transcription of many genes that are upregulated after glucose depletion or removal. Active PKA facilitates transcriptional repression by Sok2 and Ume6, prevents nuclear accumulation of Msn2 and Msn4, and inhibits the kinases Rim15 and Yak1 which activate transcription factors such as Gis1 and Hsf1 (Enjalbert et al., 2004; Gorner et al., 1998; Lee et al., 2008; Pedruzzi et al., 2000; Rubin-Bejerano et al., 1996; Shenhar and Kassir, 2001; Swinnen et al., 2006; Ward et al., 1995; Xiao and Mitchell, 2000). Notably, of the genes that

display substantially greater upregulation in ρ^+ than ρ^0 cells after glucose removal, 38.5% are predicted to be regulated by Sok2, 34.3% by Hsf1, 27.3% by Msn2, 17.5% by Msn4, and 14.4% by Ume6, according to analyses employing yeastract.com (Monteiro et al., 2008; Teixeira et al., 2006). Under normal circumstances, both Msn2 and Ume6 become hyperphosphorylated upon depletion of glucose (Garreau et al., 2000; Xiao and Mitchell, 2000). We analyzed the mobility shifts associated with phosphorylation of Msn2 and Ume6 using strains expressing TAP tagged versions of either Msn2 or Ume6 from their endogenous loci. During log growth, Msn2 and Ume6 displayed similar mobility in ρ^+ and ρ^0 cells (Figure 6.3 lanes 1 and 2 in A and B). At 3 and 4 days of growth, both Msn2 and Ume6 display mobility shifts in ρ^+ cells but not ρ^0 cells. These results are consistent with hyperactivity of PKA in ρ^0 cells for two reasons. First, Garreau *et al* have demonstrated that artificial activation of PKA at the diauxic shift blocks hyperphosphorylation of Msn2 and Msn4 (Garreau et al., 2000). Second, the Gsk3 β homologue Rim11, which is responsible for hyperphosphorylation of Ume6, is phosphorylated and inactivated by PKA (Rubin-Bejerano et al., 2004; Xiao and Mitchell, 2000).

To assess the functional significance of these results, we chose to examine protein levels of two enzymes in post-diauxic cultures: 1) acetyl-CoA synthase 1 (Acs1), which is glucose repressible, and 2) acetyl-CoA synthase 2 (Acs2), which is constitutively expressed. *ACS1* gene expression is controlled by factors known to be affected by PKA and Snf1 activities (Ciriacy, 1979; Denis, 1987; Kratzer and Schuller, 1997; Malathi et al., 1997; Rahner et al., 1999; Rubin-Bejerano et al., 1996; Xiao and Mitchell, 2000) (Figure 6.4 A). We employed strains expressing TAP tagged versions of *ACS1* or *ACS2* from their endogenous locations for these experiments. *ACS1* displays 2.4 fold greater mRNA expression in ρ^+ cells in S_C than ρ^0 cells in S_C. The *ACS1* promoter contains two URS1 sequences, which facilitate Ume6 directed repression of the gene, as well as a CSRE and an Adr1 binding site which allow transcriptional induction in the absence of glucose (Kratzer and Schuller, 1997). Three Abf1 binding sites appeared to facilitate basal expression if Ume6 is absent (Kratzer and Schuller, 1997).

In post diauxic cultures grown with glucose as the carbon source, Acs1 was robustly expressed in ρ^+ but not ρ^0 cells (Figure 6.4 B lanes 3 and 4). In contrast, protein levels of Acs2 are very

similar in ρ^+ and ρ^0 cells (Figure 6.4 B lanes 5 and 6). Therefore in addition failing to hyperphosphorylate Ume6 (Figure 6.3 B), ρ^0 cells fail to accumulate the protein product of a Ume6 regulated gene during diauxic shift.

The work of Kratzer and Schüller suggests that approximately 80% of derepressed *ACS1* transcription is mediated by Adr1 and Cat8 (Kratzer and Schuller, 1997). Both of these factors depend on Snf1 activity (Cherry et al., 1989; Ciriacy, 1979; Rahner et al., 1999). While Snf1 appears to become activated normally in glucose grown cells, there is a minor defect in phosphorylation of at least one of its substrates (Figure 6.2 A and B). Snf1 is active during proliferative growth on alternative carbon sources. Raffinose, a fermentable carbon source which does not repress Snf1 activity, was used in place of glucose to allow activation of the Snf1 pathway prior to carbon source depletion. In post-diauxic cultures, Acs1 is more abundant in ρ^0 cells grown in raffinose than in glucose (Figure 6.4, B lanes 4 and 10). The abundance of Acs1 in raffinose grown ρ^0 cells is still much lower than that of ρ^+ cells grown in raffinose or glucose (Figure 6.4, B lanes 3, 9, and 10). This again suggests that misregulation of Snf1 is not the primary regulatory

defect in ρ^0 cells. Instead, inappropriate PKA activity is likely to be the cause of defects in expression of *ACS1* in ρ^0 cells, as PKA has been seen both to promote the repression of genes by Ume6 and to antagonize the transcriptional activity of Adr1 (Cherry et al., 1989; Dombek and Young, 1997; Rubin-Bejerano et al., 2004; Xiao and Mitchell, 2000).

6.2.6 What mechanism could account for inappropriate activation of the PKA pathway in ρ^0 cells?

While the evidence presented so far is consistent with failure to down-regulate PKA activity in ρ^0 cells, the mechanism for this is not immediately evident. PKA activity is generally inactivated following a decline in intracellular cAMP concentration which allows association of Bcy1 with the catalytic subunits (Toda et al., 1987a). The decline in cAMP is preceded by deactivation of the GTP binding proteins Ras1, Ras2, and Gpa2 which all bind and activate the adenylate cyclase Cyr1 (Toda et al., 1985). Mechanisms which shift the ratio of GTP/GDP bound towards the GDP bound state deactivate GTP binding proteins. This is generally achieved through activation of the intrinsic GTPase activity of GTP binding proteins by GTPase activating proteins

(GAPs). Ira1 and Ira2 function as Ras GAPs and RGS2 as the Gpa2 GAP (Tanaka et al., 1989; Tanaka et al., 1990a; Tanaka et al., 1990b; Versele et al., 1999). Additional evidence suggests that the abundance of GTP declines as cells progress through diauxic shift, and this decreases the efficiency of exchange of GDP for GTP stimulated by the Ras GEF, Cdc25 (Cazzaniga et al., 2008; Rudoni et al., 2001). How either of these mechanisms would be affected by loss of mtDNA and respiratory capacity is uncertain.

One possibility was raised by the observations of Wang and Deschenes (2006). They noted that inhibition of respiration with sodium azide resulted in relocalization of Ras2 to the mitochondria (Wang and Deschenes, 2006). Due to the respiratory defects of p^0 cells, a similar relocalization of the Ras proteins might occur in these cells. This might sequester the Ras proteins, isolating them from the factors which normally mediate their inactivation.

Before initiating in-depth studies of Ras activation and localization, we tested if reducing Ras activity would have any effect on the defects in phosphorylation of Ume6 seen in p^0 cells. Cells were harvested from log phase cultures at 1 OD₆₀₀ and

then after 3 days and 4 days growth, to examine the mobility shift displayed by Ume6 in WT ρ^+ and ρ^0 cells and *ras2 Δ* ρ^0 cells. Ume6 shifting is evident in *ras2 Δ* ρ^0 cells even in log (Figure 6.6 A lanes 1-3). This shifting is lesser in magnitude than that displayed in WT ρ^+ cells but the mobility of Ume6 is clearly retarded in *ras2 Δ* ρ^0 cells in comparison to WT ρ^0 cells at all time points. Thus reducing Ras activity in ρ^0 cells can allow a Ume6 phosphorylation shift to occur. Since Ras1 provides some Ras activity in *ras2 Δ* cells, Ras1 may block complete shifting in *ras2 Δ* ρ^0 cells. Ras1 bears significant homology to Ras2 and is likely to be subject to similar regulation (Kataoka et al., 1984).

Direct examination of Ras2 protein levels in ρ^+ and ρ^0 cells yielded an unexpected result. Ras2 migrated as a single tight band in both ρ^+ and ρ^0 cells during log growth (Figure 6.6 B lanes 1 and 2). After 3 or 4 days growth, additional slower migrating forms of Ras2 are evident in ρ^+ but not ρ^0 cells (Figure 6.6 B lanes 3-6). Like the other mobility shifts discussed thus far, this mobility shift is likely to represent phosphorylation of the protein. The shifting behavior of Ras2 is evident when a trichloroacetic acid (TCA) precipitation procedure is used for protein preparation, but not in lysates from bead beating

procedures (data not shown). We have therefore been unable to use phosphatase treatment to test the assumption that the shift is due to phosphorylation. Other groups have previously noted advantages of TCA extraction. While studying phosphorylation of Ime1, Yona Kassir's group noted that the phosphorylation state of Ime1 was preserved much better in TCA precipitations than other methods of extraction (Rubin-Bejerano et al., 2004). Phosphorylation of the Ras proteins has been noted previously both in mammals and yeast. PKA and protein kinase C (PKC) directed phosphorylation of c-H- and c-K-ras have been detected (Saikumar et al., 1988). Ras2 can be phosphorylated by PKA *in vitro* and this inhibits Ras activity (Resnick and Racker, 1988). Phosphorylation of Ras1 and Ras2 *in vivo* has been demonstrated (Cobitz et al., 1989). In log phase, modification of Ras2 seems to occur primarily at Ser 214 (Whistler and Rine, 1997). Phenotypic analysis of strains harboring mutant forms of Ras2 which are not phosphorylated suggested that phosphorylation of Ser214 may increase signaling through the Ras-cAMP pathway (Whistler and Rine, 1997). Notably, log phase phosphorylation of Ras2 does not produce detectable shifts in mobility during gel electrophoresis (Cobitz et al., 1989). The modification of Ras2 which we witness during passage through

the diauxic shift produces clear shifts and is likely to be functionally distinct from the phosphorylation of Ras2 during log phase.

6.3 Conclusions

A study by Imoberdorf *et al.* has previously demonstrated that lowering global histone acetylation levels, via deletion of *GCN5*, increases the minimum strength of activator required to initiate transcription (Imoberdorf *et al.*, 2006). As this study used artificial recruitment of TBP to a promoter as their model for strength of activator, the actual impact of global acetylation on normal transcriptional activators and TBP recruitment is unclear (for a detailed description of the Imoberdorf study see section 1.5). At first glance, removal of glucose from cultures of ρ^+ and ρ^0 cells provided a system which had three traits that made it desirable for studying *in vivo* effects of histone acetylation on transcription. First, glucose removal initiates establishment of a new transcriptional program, allowing dynamic changes in transcription to be monitored. Second, overall histone acetylation drops rapidly during this transition in ρ^0 cells but is maintained in ρ^+ cells, allowing for comparison of

the transcriptional programs established in cells with high acetylation to that established by cells with lowered overall acetylation. Third, the alterations in histone acetylation are achieved without mutation or deletion of any of the KAT or HDAC, thereby providing a system in which overall acetylation could be modulated without mutation of any component of the transcriptional apparatus.

Our subsequent work revealed that a large portion of the transcriptional effects in this system are due to signaling abnormalities in ρ^0 cells. Although this system does not provide a suitable model for studying the varying effects of overall histone acetylation on the ability of transcriptional activators to induce transcription, transcriptional profiling of this system has revealed some interesting biology. Cells lacking mtDNA are unable to upregulate the expression of numerous glucose repressed genes. Various pieces of evidence point towards this defect being a result of inappropriate maintenance of PKA activity after glucose removal in ρ^0 cells. First, a substantial portion of the genes which ρ^0 cells fail to upregulate are regulated by transcription factors that are regulated by PKA. Second, hyperphosphorylation of Msn2 and Ume6 after removal

of glucose is blocked in ρ^0 cells. PKA activity is known to prevent hyperphosphorylation of these proteins (Gorner et al., 1998; Malathi et al., 1997; Rubin-Bejerano et al., 1996). Third, our results suggest that regulation of Ras2 may be aberrant in ρ^0 cells.

We do not exclude the possibility that aberrations in other signaling pathways may contribute to these defects, but we suspect PKA activity is likely to be the dominant contributor. The TOR kinases, whose activities appear to be responsive to nitrogen sources, are also known to regulate the subcellular localization of Msn2 and Msn4 (Beck and Hall, 1999; Santhanam et al., 2004). Studies of meiosis, employing respiratory deficient cells, suggest that TOR signaling is not likely to be the principal pathway causing defects in ρ^0 cells, however. Specifically Jambhekar and Amon (2008) found that inhibition of TOR signaling, with the macrolide antibiotic rapamycin, induced expression of an *IME1*-LacZ reporter construct in wild type cells but not in respiratory deficient *pet100* Δ cells. This suggests that a pathway other than TOR which is responsible either for transcriptional induction or repression *IME1* is defective in respiratory deficient cells. Notably *IME1* expression is controlled

by Msn2/4 and Sok2, which both are regulated by PKA (Shenhar and Kassir, 2001). Further investigation will be required to elucidate the mechanistic cause of the signaling defects which we have described in this chapter.

Table 6.1 Gene Ontology (GO) terms overrepresented in groups of genes that display a twofold or greater change in expression level.

2 Fold List	GO term	p value
ρ^+ log > ρ^+ S_C genes repressed by glucose removal in ρ^+ cells	Response to H ₂ O ₂	0.00236
ρ^+ log > ρ^0 log Genes repressed in ρ^0 in comparison to ρ^+ cells in SDC	Sexual reproduction	1.38×10^{-6}
	Conjugation with cell fussion	1.38×10^{-6}
	Reproductive physiological process	1.38×10^{-6}
	Reproductive cellular process	1.38×10^{-6}
	Conjugation	1.38×10^{-6}
ρ^+ S_C > ρ^+ log genes induced by glucose removal in ρ^+ cells	Energy derivation by oxidation of organic compounds	7.68×10^{-8}
	Generation of precursor metabolites and energy	1.03×10^{-7}
	Cellular respiration	4.96×10^{-6}
	Aerobic respiration	1.27×10^{-5}
	Organic acid metabolism	3.09×10^{-5}
	Carboxylic acid metabolism	3.09×10^{-5}
ρ^+ S_C > ρ^0 S_C genes repressed in ρ^0 in comparison to ρ^+ cells after glucose removal	Generation of precursor metabolites and energy	1.14×10^{-6}
	Cellular respiration	2.31×10^{-5}
	Energy derivation by oxidation of organic compounds	2.87×10^{-5}
	Aerobic respiration	5.69×10^{-5}
ρ^0 log > ρ^+ log genes induced in ρ^0 in comparison to ρ^+ cells in SDC	Vitamin B6 metabolism	2.18×10^{-5}
	Pyridoxine metabolism	2.18×10^{-5}
	Response to drug	3.37×10^{-4}
	NADH oxidation	5.14×10^{-4}
ρ^0 log > ρ^0 S_C genes repressed by glucose removal in ρ^0 cells	Regulation of CDK activity	8.6×10^{-4}
ρ^0 S_C > ρ^+ S_C genes induced in ρ^0 in comparison to ρ^+ cells after glucose removal	DNA metabolism	9.74×10^{-5}
	Organell organization and biogenesis	4.09×10^{-4}
ρ^0 S_C > ρ^0 log genes induced by glucose removal in ρ^0 cells	Mono saccharide transport	4.39×10^{-4}
	Hexose transport	4.39×10^{-4}

Note: log (mRNAs from 1 OD₆₀₀ SDC cultures). S_C (mRNAs from 1 OD₆₀₀ cultures after 4 hours in S_C). > (greater than).

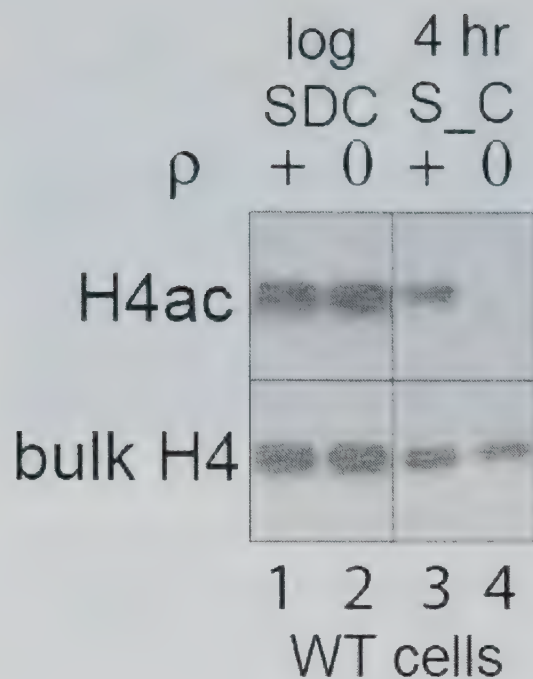


Figure 6.1 Rapid loss of overall histone H4 acetylation after glucose removal in ρ^0 cells.

SDC cultures of wild type ρ^+ and ρ^0 cells were grown to 1 OD₆₀₀ then collected and washed in the same media without glucose (S_C). They were next resuspended in S_C and incubated for 4hrs at room temperature with constant shaking.

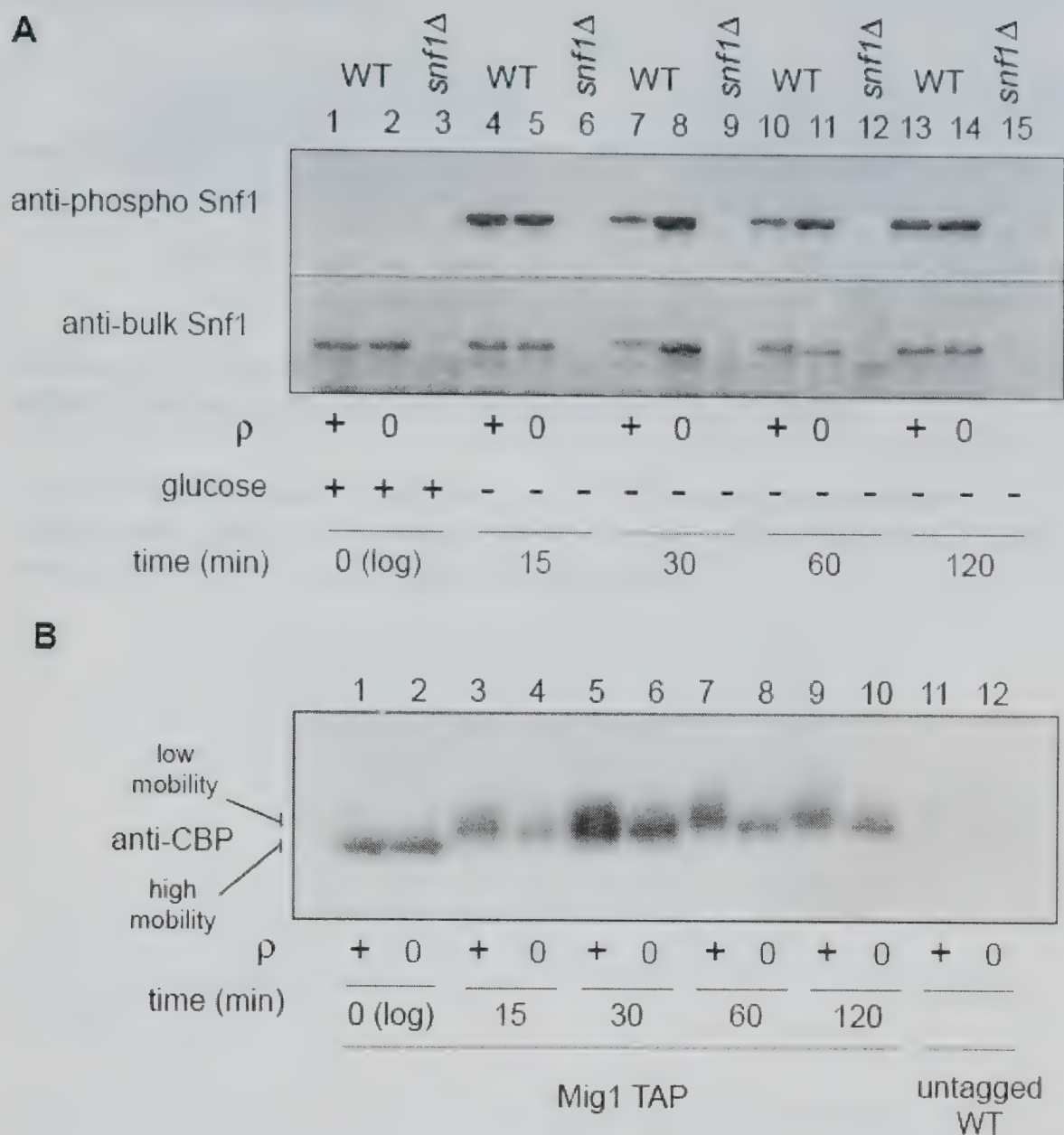


Figure 6.2 Activating phosphorylation of Snf1 occurs normally in ρ^0 cells but phosphorylation of the Snf1 substrate Mig1 is attenuated.

SDC cultures of wild type ρ^+ and ρ^0 cells were grown to 1 OD₆₀₀ (log) collected and washed in the same media without glucose (S_C), then resuspended in S_C at 1 OD₆₀₀ and incubated at room temperature.

(A) Snf1 phosphorylated at Thr210 was detected using an antibody raised against a phospho-peptide corresponding to the residues surrounding Thr172 of Human AMPK α (anti-phospho Snf1) (Cell Signaling). Bulk Snf1 was detected with a antibody raised against a C-terminal peptide from Snf1 (Santa cruz biotechnology, sc-15622).

(B) TAP-tagged Mig1 expressed from its endogenous location was detected with an anti-CBP antibody.

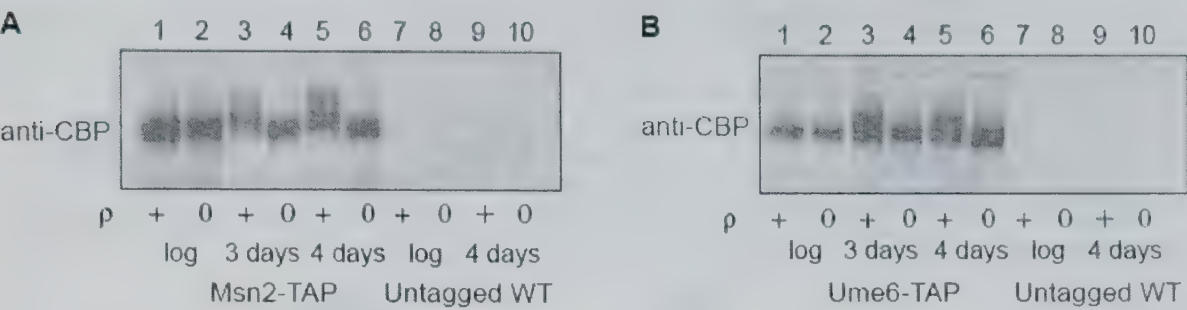


Figure 6.3 Hyperphosphorylation of Msn2 and Ume6, after glucose depletion, is blocked in ρ^0 cells.

(A) & (B) YPD cultures of wildtype ρ^+ and ρ^0 cells were grown at room temperature. Cells were harvested for analysis at 1 OD₆₀₀ (log) then 72hrs (3 days) and 96 hrs(4 days) after the initial harvest.

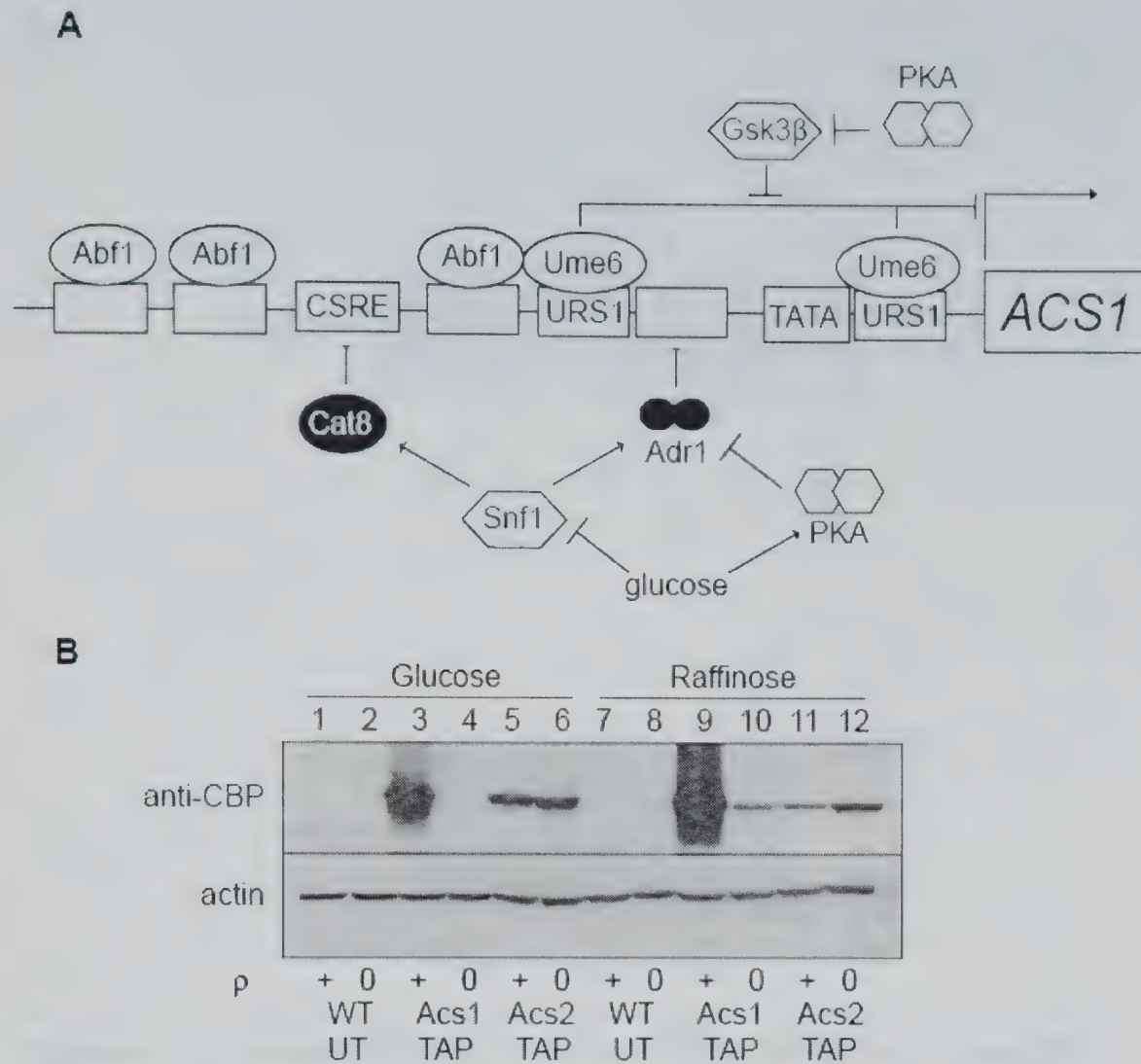


Figure 6.4 Stationary phase ρ^0 cells fail to accumulate Acs1p.

(A) Architecture of the *ACS1* promoter, and Snf1 and PKA directed regulation. (B) Wild type untagged (UT) and TAP-tagged *Acs1* or *Acs2* ρ^+ and ρ^0 cells were grown in YPD (glucose) or YPR (raffinose) for 7 days then harvested for Western analysis. An anti-CBP antibody was used to detect TAP-tagged versions of *Acs1* or *Acs2*.

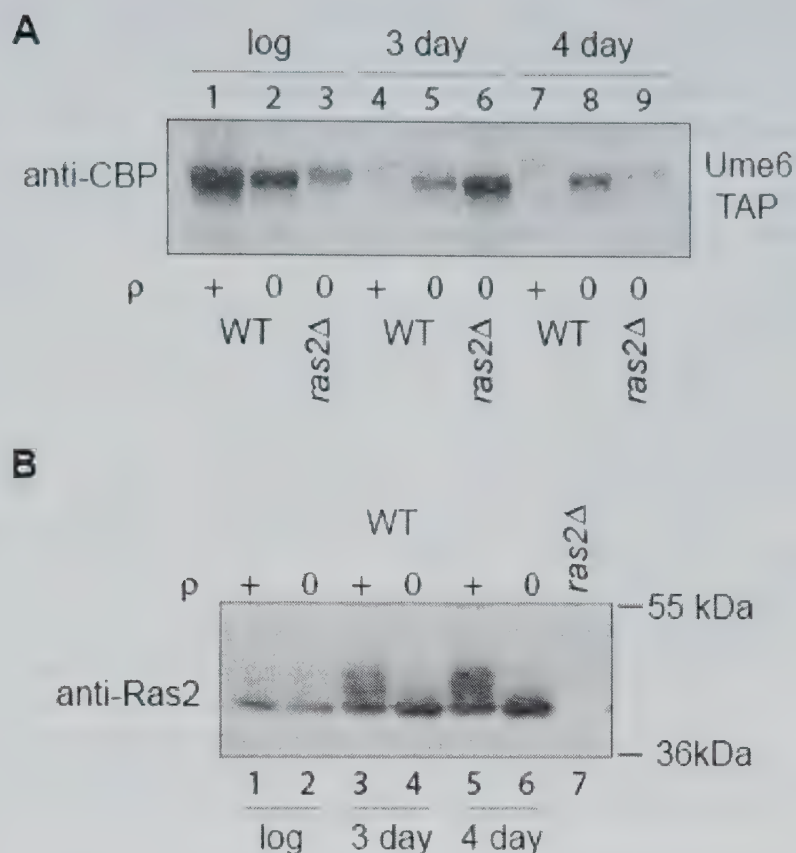


Figure 6.6 Ume6 displays a mobility shift in ρ^0 *ras2* Δ cells but not wild type ρ^0 cells. During nutrient depletion, Ras2p undergoes a mobility shift in ρ^+ cells but not ρ^0 cells.

YPD cultures of WT ρ^+ and ρ^0 cells were grown at room temperature. Cells were harvested for analysis at 1 OD₆₀₀ (log) then 72hrs (3days) and 96 hrs (4days) after the initial harvest.

(A) TAP-tagged Ume6 was expressed from its endogenous location and detected using an anti-CBP antibody.

(B) Ras2 was detected with an antibody raised against a peptide from the C-terminus of Ras2 (Santa cruz biotechnology, sc-6759).

6.4 References

- Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*, **25**, 25-29.
- Beck, T. and Hall, M.N. (1999) The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature*, **402**, 689-692.
- Broek, D., Samiy, N., Fasano, O., Fujiyama, A., Tamanoi, F., Northup, J. and Wigler, M. (1985) Differential activation of yeast adenylate cyclase by wild-type and mutant RAS proteins. *Cell*, **41**, 763-769.
- Carrozza, M.J., Florens, L., Swanson, S.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005) Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. *Biochim Biophys Acta*, **1731**, 77-87; discussion 75-76.
- Cazzaniga, P., Pescini, D., Besozzi, D., Mauri, G., Colombo, S. and Martegani, E. (2008) Modeling and stochastic simulation of the Ras/cAMP/PKA pathway in the yeast *Saccharomyces cerevisiae* evidences a key regulatory function for intracellular guanine nucleotides pools. *J Biotechnol*, **133**, 377-385.
- Celenza, J.L. and Carlson, M. (1989) Mutational analysis of the *Saccharomyces cerevisiae* SNF1 protein kinase and evidence for functional interaction with the SNF4 protein. *Mol Cell Biol*, **9**, 5034-5044.
- Celenza, J.L., Eng, F.J. and Carlson, M. (1989) Molecular analysis of the SNF4 gene of *Saccharomyces cerevisiae*: evidence for physical association of the SNF4 protein with the SNF1 protein kinase. *Mol Cell Biol*, **9**, 5045-5054.
- Cherry, J.R., Johnson, T.R., Dollard, C., Shuster, J.R. and Denis, C.L. (1989) Cyclic AMP-dependent protein kinase phosphorylates and inactivates the yeast transcriptional activator ADR1. *Cell*, **56**, 409-419.
- Ciriacy, M. (1979) Isolation and characterization of further cis- and trans-acting regulatory elements involved in the synthesis of glucose-repressible alcohol dehydrogenase (ADHII) in *Saccharomyces cerevisiae*. *Mol Gen Genet*, **176**, 427-431.
- Cobitz, A.R., Yim, E.H., Brown, W.R., Perou, C.M. and Tamanoi, F. (1989) Phosphorylation of RAS1 and RAS2 proteins in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*, **86**, 858-862.
- Denis, C.L. (1987) The effects of ADR1 and CCR1 gene dosage on the regulation of the glucose-repressible alcohol dehydrogenase from *Saccharomyces cerevisiae*. *Mol Gen Genet*, **208**, 101-106.
- DeVit, M.J. and Johnston, M. (1999) The nuclear exportin Msn5 is required for nuclear export of the Mig1 glucose repressor of *Saccharomyces cerevisiae*. *Curr Biol*, **9**, 1231-1241.
- Dombek, K.M. and Young, E.T. (1997) Cyclic AMP-dependent protein kinase inhibits ADH2 expression in part by decreasing expression of the transcription factor gene ADR1. *Mol Cell Biol*, **17**, 1450-1458.

- Enjalbert, B., Parrou, J.L., Teste, M.A. and Francois, J. (2004) Combinatorial control by the protein kinases PKA, PHO85 and SNF1 of transcriptional induction of the *Saccharomyces cerevisiae* GSY2 gene at the diauxic shift. *Mol Genet Genomics*, **271**, 697-708.
- Enjalbert, B., Parrou, J.L., Vincent, O. and Francois, J. (2000) Mitochondrial respiratory mutants of *Saccharomyces cerevisiae* accumulate glycogen and readily mobilize it in a glucose-depleted medium. *Microbiology*, **146 (Pt 10)**, 2685-2694.
- Epstein, C.B., Waddle, J.A., Hale, W.t., Dave, V., Thornton, J., Macatee, T.L., Garner, H.R. and Butow, R.A. (2001) Genome-wide responses to mitochondrial dysfunction. *Mol Biol Cell*, **12**, 297-308.
- Erickson, J.R. and Johnston, M. (1993) Genetic and molecular characterization of GAL83: its interaction and similarities with other genes involved in glucose repression in *Saccharomyces cerevisiae*. *Genetics*, **135**, 655-664.
- Garreau, H., Hasan, R.N., Renault, G., Estruch, F., Boy-Marcotte, E. and Jacquet, M. (2000) Hyperphosphorylation of Msn2p and Msn4p in response to heat shock and the diauxic shift is inhibited by cAMP in *Saccharomyces cerevisiae*. *Microbiology*, **146 (Pt 9)**, 2113-2120.
- Gorner, W., Durchschlag, E., Martinez-Pastor, M.T., Estruch, F., Ammerer, G., Hamilton, B., Ruis, H. and Schuller, C. (1998) Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev*, **12**, 586-597.
- Hawley, S.A., Boudeau, J., Reid, J.L., Mustard, K.J., Udd, L., Makela, T.P., Alessi, D.R. and Hardie, D.G. (2003) Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol*, **2**, 28.
- Hawley, S.A., Davison, M., Woods, A., Davies, S.P., Beri, R.K., Carling, D. and Hardie, D.G. (1996) Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J Biol Chem*, **271**, 27879-27887.
- Hawley, S.A., Pan, D.A., Mustard, K.J., Ross, L., Bain, J., Edelman, A.M., Frenguelli, B.G. and Hardie, D.G. (2005) Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab*, **2**, 9-19.
- Hedbacker, K., Hong, S.P. and Carlson, M. (2004a) Pak1 protein kinase regulates activation and nuclear localization of Snf1-Gal83 protein kinase. *Mol Cell Biol*, **24**, 8255-8263.
- Hedbacker, K., Townley, R. and Carlson, M. (2004b) Cyclic AMP-dependent protein kinase regulates the subcellular localization of Snf1-Sip1 protein kinase. *Mol Cell Biol*, **24**, 1836-1843.
- Hong, S.P., Leiper, F.C., Woods, A., Carling, D. and Carlson, M. (2003) Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc Natl Acad Sci U S A*, **100**, 8839-8843.
- Hurley, R.L., Anderson, K.A., Franzone, J.M., Kemp, B.E., Means, A.R. and Witters, L.A. (2005) The Ca²⁺/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem*, **280**, 29060-29066.

- Imoberdorf, R.M., Topalidou, I. and Strubin, M. (2006) A role for gcn5-mediated global histone acetylation in transcriptional regulation. *Mol Cell Biol*, **26**, 1610-1616.
- Jambhekar, A. and Amon, A. (2008) Control of meiosis by respiration. *Curr Biol*, **18**, 969-975.
- Jiang, R. and Carlson, M. (1996) Glucose regulates protein interactions within the yeast SNF1 protein kinase complex. *Genes Dev*, **10**, 3105-3115.
- Katan-Khaykovich, Y. and Struhl, K. (2002) Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes Dev*, **16**, 743-752.
- Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J. and Wigler, M. (1984) Genetic analysis of yeast RAS1 and RAS2 genes. *Cell*, **37**, 437-445.
- Kraakman, L., Lemaire, K., Ma, P., Teunissen, A.W., Donaton, M.C., Van Dijck, P., Winderickx, J., de Winde, J.H. and Thevelein, J.M. (1999) A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. *Mol Microbiol*, **32**, 1002-1012.
- Kratzer, S. and Schuller, H.J. (1997) Transcriptional control of the yeast acetyl-CoA synthetase gene, ACS1, by the positive regulators CAT8 and ADR1 and the pleiotropic repressor UME6. *Mol Microbiol*, **26**, 631-641.
- Lee, P., Cho, B.R., Joo, H.S. and Hahn, J.S. (2008) Yeast Yak1 kinase, a bridge between PKA and stress-responsive transcription factors, Hsf1 and Msn2/Msn4. *Mol Microbiol*, **70**, 882-895.
- Lemaire, K., Van de Velde, S., Van Dijck, P. and Thevelein, J.M. (2004) Glucose and sucrose act as agonist and mannose as antagonist ligands of the G protein-coupled receptor Gpr1 in the yeast *Saccharomyces cerevisiae*. *Mol Cell*, **16**, 293-299.
- Liu, Z. and Butow, R.A. (2006) Mitochondrial retrograde signaling. *Annu Rev Genet*, **40**, 159-185.
- Malathi, K., Xiao, Y. and Mitchell, A.P. (1997) Interaction of yeast repressor-activator protein Ume6p with glycogen synthase kinase 3 homolog Rim11p. *Mol Cell Biol*, **17**, 7230-7236.
- Matsumoto, K., Uno, I., Oshima, Y. and Ishikawa, T. (1982) Isolation and characterization of yeast mutants deficient in adenylate cyclase and cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A*, **79**, 2355-2359.
- Mbonyi, K., Beullens, M., Detremmerie, K., Geerts, L. and Thevelein, J.M. (1988) Requirement of one functional RAS gene and inability of an oncogenic ras variant to mediate the glucose-induced cyclic AMP signal in the yeast *Saccharomyces cerevisiae*. *Mol Cell Biol*, **8**, 3051-3057.
- McCartney, R.R. and Schmidt, M.C. (2001) Regulation of Snf1 kinase. Activation requires phosphorylation of threonine 210 by an upstream kinase as well as a distinct step mediated by the Snf4 subunit. *J Biol Chem*, **276**, 36460-36466.
- Momcilovic, M., Hong, S.P. and Carlson, M. (2006) Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. *J Biol Chem*, **281**, 25336-25343.
- Monteiro, P.T., Mendes, N.D., Teixeira, M.C., d'Orey, S., Tenreiro, S., Mira, N.P., Pais, H., Francisco, A.P., Carvalho, A.M., Lourenco, A.B., Sa-

- Correia, I., Oliveira, A.L. and Freitas, A.T. (2008) YEASTRACT-DISCOVERER: new tools to improve the analysis of transcriptional regulatory associations in *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **36**, D132-136.
- Nath, N., McCartney, R.R. and Schmidt, M.C. (2003) Yeast Pak1 kinase associates with and activates Snf1. *Mol Cell Biol*, **23**, 3909-3917.
- Orlova, M., Kanter, E., Krakovich, D. and Kuchin, S. (2006) Nitrogen availability and TOR regulate the Snf1 protein kinase in *Saccharomyces cerevisiae*. *Eukaryot Cell*, **5**, 1831-1837.
- Ostling, J. and Ronne, H. (1998) Negative control of the Mig1p repressor by Snf1p-dependent phosphorylation in the absence of glucose. *Eur J Biochem*, **252**, 162-168.
- Papamichos-Chronakis, M., Conlan, R.S., Gounalaki, N., Copf, T. and Tzamarias, D. (2000) Hrs1/Med3 is a Cyc8-Tup1 corepressor target in the RNA polymerase II holoenzyme. *J Biol Chem*, **275**, 8397-8403.
- Pedruzzi, I., Burckert, N., Egger, P. and De Virgilio, C. (2000) *Saccharomyces cerevisiae* Ras/cAMP pathway controls post-diauxic shift element-dependent transcription through the zinc finger protein Gis1. *Embo J*, **19**, 2569-2579.
- Peeters, T., Louwet, W., Gelade, R., Nauwelaers, D., Thevelein, J.M. and Versele, M. (2006) Kelch-repeat proteins interacting with the Galpha protein Gpa2 bypass adenylate cyclase for direct regulation of protein kinase A in yeast. *Proc Natl Acad Sci U S A*, **103**, 13034-13039.
- Rahner, A., Hiesinger, M. and Schuller, H.J. (1999) Deregulation of gluconeogenic structural genes by variants of the transcriptional activator Cat8p of the yeast *Saccharomyces cerevisiae*. *Mol Microbiol*, **34**, 146-156.
- Resnick, R.J. and Racker, E. (1988) Phosphorylation of the RAS2 gene product by protein kinase A inhibits the activation of yeast adenylate cyclase. *Proc Natl Acad Sci U S A*, **85**, 2474-2478.
- Rolland, F., De Winder, J.H., Lemaire, K., Boles, E., Thevelein, J.M. and Winderickx, J. (2000) Glucose-induced cAMP signalling in yeast requires both a G-protein coupled receptor system for extracellular glucose detection and a separable hexose kinase-dependent sensing process. *Mol Microbiol*, **38**, 348-358.
- Rubin-Bejerano, I., Mandel, S., Robzyk, K. and Kassir, Y. (1996) Induction of meiosis in *Saccharomyces cerevisiae* depends on conversion of the transcriptional repressor Ume6 to a positive regulator by its regulated association with the transcriptional activator Ime1. *Mol Cell Biol*, **16**, 2518-2526.
- Rubin-Bejerano, I., Sagee, S., Friedman, O., Pnueli, L. and Kassir, Y. (2004) The in vivo activity of Ime1, the key transcriptional activator of meiosis-specific genes in *Saccharomyces cerevisiae*, is inhibited by the cyclic AMP/protein kinase A signal pathway through the glycogen synthase kinase 3-beta homolog Rim11. *Mol Cell Biol*, **24**, 6967-6979.
- Rudoni, S., Colombo, S., Coccetti, P. and Martegani, E. (2001) Role of guanine nucleotides in the regulation of the Ras/cAMP pathway in *Saccharomyces cerevisiae*. *Biochim Biophys Acta*, **1538**, 181-189.
- Saikumar, P., Ulsh, L.S., Clanton, D.J., Huang, K.P. and Shih, T.Y. (1988) Novel phosphorylation of c-ras p21 by protein kinases. *Oncogene Res*, **3**, 213-222.

- Sanders, M.J., Grondin, P.O., Hegarty, B.D., Snowden, M.A. and Carling, D. (2007) Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J*, **403**, 139-148.
- Santhanam, A., Hartley, A., Duvel, K., Broach, J.R. and Garrett, S. (2004) PP2A phosphatase activity is required for stress and Tor kinase regulation of yeast stress response factor Msn2p. *Eukaryot Cell*, **3**, 1261-1271.
- Sanz, P., Alms, G.R., Haystead, T.A. and Carlson, M. (2000) Regulatory interactions between the Reg1-Glc7 protein phosphatase and the Snf1 protein kinase. *Mol Cell Biol*, **20**, 1321-1328.
- Schuller, H.J. (2003) Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. *Curr Genet*, **43**, 139-160.
- Shahbazian, M.D. and Grunstein, M. (2007) Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem*, **76**, 75-100.
- Shaw, R.J., Kosmatka, M., Bardeesy, N., Hurley, R.L., Witters, L.A., DePinho, R.A. and Cantley, L.C. (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci U S A*, **101**, 3329-3335.
- Shenhar, G. and Kassir, Y. (2001) A positive regulator of mitosis, Sok2, functions as a negative regulator of meiosis in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **21**, 1603-1612.
- Smith, A., Ward, M.P. and Garrett, S. (1998) Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation. *Embo J*, **17**, 3556-3564.
- Smith, F.C., Davies, S.P., Wilson, W.A., Carling, D. and Hardie, D.G. (1999) The SNF1 kinase complex from *Saccharomyces cerevisiae* phosphorylates the transcriptional repressor protein Mig1p in vitro at four sites within or near regulatory domain 1. *FEBS Lett*, **453**, 219-223.
- Sutherland, C.M., Hawley, S.A., McCartney, R.R., Leech, A., Stark, M.J., Schmidt, M.C. and Hardie, D.G. (2003) Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex. *Curr Biol*, **13**, 1299-1305.
- Swinnen, E., Wanke, V., Roosen, J., Smets, B., Dubouloz, F., Pedruzzi, I., Cameroni, E., De Virgilio, C. and Winderickx, J. (2006) Rim15 and the crossroads of nutrient signalling pathways in *Saccharomyces cerevisiae*. *Cell Div*, **1**, 3.
- Tanaka, K., Matsumoto, K. and Toh, E.A. (1989) IRA1, an inhibitory regulator of the RAS-cyclic AMP pathway in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **9**, 757-768.
- Tanaka, K., Nakafuku, M., Satoh, T., Marshall, M.S., Gibbs, J.B., Matsumoto, K., Kaziro, Y. and Toh-e, A. (1990a) *S. cerevisiae* genes IRA1 and IRA2 encode proteins that may be functionally equivalent to mammalian ras GTPase activating protein. *Cell*, **60**, 803-807.
- Tanaka, K., Nakafuku, M., Tamanoi, F., Kaziro, Y., Matsumoto, K. and Toh-e, A. (1990b) IRA2, a second gene of *Saccharomyces cerevisiae* that encodes a protein with a domain homologous to mammalian ras GTPase-activating protein. *Mol Cell Biol*, **10**, 4303-4313.
- Teixeira, M.C., Monteiro, P., Jain, P., Tenreiro, S., Fernandes, A.R., Mira, N.P., Alenquer, M., Freitas, A.T., Oliveira, A.L. and Sa-Correia, I. (2006) The YEASTRACT database: a tool for the analysis of transcription

- regulatory associations in *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **34**, D446-451.
- Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J.D., McMullen, B., Hurwitz, M., Krebs, E.G. and Wigler, M. (1987a) Cloning and characterization of BCY1, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **7**, 1371-1377.
- Toda, T., Cameron, S., Sass, P., Zoller, M. and Wigler, M. (1987b) Three different genes in *S. cerevisiae* encode the catalytic subunits of the cAMP-dependent protein kinase. *Cell*, **50**, 277-287.
- Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K. and Wigler, M. (1985) In yeast, RAS proteins are controlling elements of adenylate cyclase. *Cell*, **40**, 27-36.
- Traven, A., Wong, J.M., Xu, D., Sopta, M. and Ingles, C.J. (2001) Interorganellar communication. Altered nuclear gene expression profiles in a yeast mitochondrial dna mutant. *J Biol Chem*, **276**, 4020-4027.
- Treitel, M.A. and Carlson, M. (1995) Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein. *Proc Natl Acad Sci U S A*, **92**, 3132-3136.
- Treitel, M.A., Kuchin, S. and Carlson, M. (1998) Snf1 protein kinase regulates phosphorylation of the Mig1 repressor in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **18**, 6273-6280.
- Tu, J. and Carlson, M. (1995) REG1 binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae*. *Embo J*, **14**, 5939-5946.
- Tzamarias, D. and Struhl, K. (1995) Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. *Genes Dev*, **9**, 821-831.
- Versele, M., de Winde, J.H. and Thevelein, J.M. (1999) A novel regulator of G protein signalling in yeast, Rgs2, downregulates glucose-activation of the cAMP pathway through direct inhibition of Gpa2. *Embo J*, **18**, 5577-5591.
- Vincent, O. and Carlson, M. (1998) Sip4, a Snf1 kinase-dependent transcriptional activator, binds to the carbon source-responsive element of gluconeogenic genes. *Embo J*, **17**, 7002-7008.
- Vincent, O., Townley, R., Kuchin, S. and Carlson, M. (2001) Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism. *Genes Dev*, **15**, 1104-1114.
- Vogelauer, M., Wu, J., Suka, N. and Grunstein, M. (2000) Global histone acetylation and deacetylation in yeast. *Nature*, **408**, 495-498.
- Wang, G. and Deschenes, R.J. (2006) Plasma membrane localization of Ras requires class C Vps proteins and functional mitochondria in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **26**, 3243-3255.
- Ward, M.P. and Garrett, S. (1994) Suppression of a yeast cyclic AMP-dependent protein kinase defect by overexpression of SOK1, a yeast gene exhibiting sequence similarity to a developmentally regulated mouse gene. *Mol Cell Biol*, **14**, 5619-5627.
- Ward, M.P., Gimeno, C.J., Fink, G.R. and Garrett, S. (1995) SOK2 may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. *Mol Cell Biol*, **15**, 6854-6863.

- Whistler, J.L. and Rine, J. (1997) Ras2 and Ras1 protein phosphorylation in *Saccharomyces cerevisiae*. *J Biol Chem*, **272**, 18790-18800.
- Williams, R.M., Primig, M., Washburn, B.K., Winzeler, E.A., Bellis, M., Sarrauste de Menthier, C., Davis, R.W. and Esposito, R.E. (2002) The Ume6 regulon coordinates metabolic and meiotic gene expression in yeast. *Proc Natl Acad Sci U S A*, **99**, 13431-13436.
- Wilson, W.A., Hawley, S.A. and Hardie, D.G. (1996) Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. *Curr Biol*, **6**, 1426-1434.
- Woods, A., Dickerson, K., Heath, R., Hong, S.P., Momcilovic, M., Johnstone, S.R., Carlson, M. and Carling, D. (2005) Ca²⁺/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab*, **2**, 21-33.
- Woods, A., Johnstone, S.R., Dickerson, K., Leiper, F.C., Fryer, L.G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M. and Carling, D. (2003) LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol*, **13**, 2004-2008.
- Xiao, Y. and Mitchell, A.P. (2000) Shared roles of yeast glycogen synthase kinase 3 family members in nitrogen-responsive phosphorylation of meiotic regulator Ume6p. *Mol Cell Biol*, **20**, 5447-5453.
- Xue, Y., Battle, M. and Hirsch, J.P. (1998) GPR1 encodes a putative G protein-coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway. *Embo J*, **17**, 1996-2007.
- Yang, R., Chun, K.T. and Wek, R.C. (1998) Mitochondrial respiratory mutants in yeast inhibit glycogen accumulation by blocking activation of glycogen synthase. *J Biol Chem*, **273**, 31337-31344.
- Yang, X., Hubbard, E.J. and Carlson, M. (1992) A protein kinase substrate identified by the two-hybrid system. *Science*, **257**, 680-682.
- Yang, X., Jiang, R. and Carlson, M. (1994) A family of proteins containing a conserved domain that mediates interaction with the yeast SNF1 protein kinase complex. *Embo J*, **13**, 5878-5886.
- Young, E.T., Dombek, K.M., Tachibana, C. and Ideker, T. (2003) Multiple pathways are co-regulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8. *J Biol Chem*, **278**, 26146-26158.
- Zaman, S., Lippman, S.I., Zhao, X. and Broach, J.R. (2008) How *Saccharomyces* Responds to Nutrients. *Annu Rev Genet*.

Chapter 7: Discussion

Material in this chapter has been published:

Friis, R.M. and Schultz, M.C. (2009) Untargeted tail acetylation of histones in chromatin: lessons from yeast. *Biochem Cell Biol*, **87**, 107-116.

Friis, R.M., Wu, B.P., Reinke, S.N., Hockman, D.J., Sykes, B.D. and Schultz, M.C. (2009b) A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. *Nucleic Acids Res*, **37**, 3969-3980.

A major ongoing effort in biology is focused on determining if there are causal links between physiological fluctuations in metabolism and the activity of other biochemical modules of cell function (Ladurner, 2006). Such links between carbon metabolism and NAD⁺-dependent HDACs are now well established (Grubisha et al., 2005). Here we provide the first evidence that physiological regulation of histone acetylation can also depend on direct metabolic control of KAT activity.

7.1 Glucose induction of overall H3/H4 acetylation does not depend upon replication coupled acetylation

During active proliferation, acetylation of H3 K9 and H4 K5/12 increases in concert with replication in S phase, and is elevated at active genes, usually because these are sites to which targeted KATs are recruited (Shahbazian and Grunstein, 2007). Since H3 K9 and H4 K5/8/12 are acetylated in glucose-fed cells, we initially expected histone modification events triggered by glucose to be coupled to replication and/or transcriptional activation. Our results rule out coupling of acetylation to replication, at least by the mechanism that

predominates in actively proliferating cells. In normally cycling cells, H3/H4 acetylation and bulk histone expression increase in parallel during S phase (Berndsen et al., 2008). In SP cells H3/H4 acetylation increases following glucose refeeding but bulk histone expression does not. Furthermore, when increased histone acetylation is already clearly evident, glucose refeeding does not trigger accumulation of two key cyclins that are expressed during the G1 to S-phase transition (Figure 2.4B). These findings, and the results of flow cytometry, pharmacological and metabolic labelling experiments, strongly suggest that glucose induction of overall histone acetylation in SP cells depends very little, if at all, on the mechanism that couples acetylation to replication in normally cycling cells.

7.2 Glucose induction of untargeted KAT activity

Glucose induction of overall H3 K9, 18, 27 and H4 K5, 8, 12 acetylation in SP cells depends mainly on the activity of Gcn5 and Esa1, respectively. How does glucose upregulate Gcn5-/Esa1-dependent histone acetylation in SP cells? As part of our effort to answer this question, we assessed the relative contribution of targeted and untargeted KAT activity to the stimulation of acetylation. Given that hundreds of mRNAs are

induced when SP cells are refed glucose (Liko et al., 2007), targeted KAT activity associated with induction of glucose-regulated genes was expected to account for most of the increase in acetylation triggered by glucose. Surprisingly, this is not the case: Esa1-dependent acetylation of H4 can largely be attributed to picNuA4, which is specialized for untargeted acetylation (Boudreault et al., 2003), and Gcn5-dependent H3 acetylation by SAGA (or a closely related complex) does not require SAGA subunits that are known or most likely to target it to genes in the course of transcriptional activation (Brown et al., 2001; Pray-Grant et al., 2005).

A role for SAGA in untargeted acetylation might seem surprising in view of its well-established function in locus-specific histone modification. It is noteworthy, however, that all Gcn5 complexes including SAGA readily acetylate chromatin by an untargeted mechanism *in vitro* (Allard et al., 1999; Grant et al., 1997). Therefore our evidence for untargeted acetylation by SAGA *in vivo* is consistent with its demonstrated substrate specificity.

Our finding that the acetylation state of H3 and H4 in SP cells is largely controlled by untargeted KATs is consistent with

published evidence that untargeted KATs have pervasive effects on chromatin acetylation in log phase (Imoberdorf et al., 2006; Katan-Khaykovich and Struhl, 2002; Kuo et al., 2000; Reid et al., 2000; Vogelauer et al., 2000).

Prior to our work, little was known about the physiological regulation of untargeted KAT activity. No previous studies have shown that untargeted KAT activity ever fluctuates in a way that might explain physiological changes in untargeted (global) or overall (targeted + untargeted) acetylation. In mammalian cells, physiological regulation of overall histone acetylation can occur by a replication-independent mechanism (see Section 1.11). Clearly, regulation of untargeted KAT activity might contribute to the physiological regulation of overall histone acetylation state in these cases. The physiological situations in which regulation of untargeted acetylation occur are probably not limited to yeast.

7.3 Chromatin interactions of KATs and HDACs that contribute to untargeted acetylation and deacetylation

We know from biochemical studies that numerous KATs and HDACs, whether they include targeting subunits or not, can act on chromatin in an untargeted fashion (Carrozza et al., 2005;

Grant et al., 1997; Sanchez del Pino et al., 1994; Sklenar and Parthun, 2004; Tong et al., 1998). How do such enzymes associate with chromatin substrates in the course of untargeted acetylation/deacetylation reactions?

Untargeted interactions of the heterotypic KAT/HDAC complexes with chromatin are likely to depend on subunits of these complexes that are associated with the catalytic subunit. Such interactions might involve unmodified histone tails, or tails with particular patterns of modification such as found in the vicinity of genes. The non-catalytic subunits that comprise heterotypic KATs/HDACs contain domains which may facilitate interaction with modified or unmodified residues in the histone tails (Table 1.1). Among these are acetyl-lysine interacting bromodomains, chromodomains and PHD finger domains that can interact with methylated residues, and SANT domains which may interact with unmodified histone tails (Shahbazian and Grunstein, 2007). Any of these domains could allow transient interaction of complexes with any region that contains an appropriate modification.

The various KAT/HDAC complexes differ significantly with regard to their content of potential histone-interacting domains.

One could therefore argue that the total complement of KATs/HDACs effectively allows for continual untargeted modification of the acetylation state of euchromatic nucleosomes regardless of local chromatin architectures. The individual contribution of a KAT/HDAC complex to global acetylation would then depend on two factors: 1) its relative abundance, and 2) the spectrum of modified histones with which it could interact (which is partly a function of the number and type of histone interacting domains it contains). If the interaction requires a very specific pattern of modifications, as has been demonstrated for the chromodomain proteins Eaf3 and Chd1 and the PHD protein Rco1 (Li et al., 2007), then the contribution of a particular KAT/HDAC to 'untargeted' chromatin modification will depend on the genome-wide distribution of the pattern.

Several marks that are likely distributed throughout euchromatin could facilitate untargeted acetylation. Methylation of H3 K4 is important for the maintenance of euchromatin because it helps to prevent association of the silencing enzyme Sir2 with regions outside specific silenced domains (Venkatasubrahmanyam et al., 2007). In view of this function, H3K4me is expected to be found at physiologically significant

levels throughout substantial regions of euchromatin. The same might hold for acetylation of H4K16 and methylation of H3K79, which are also involved in establishing the boundaries of silenced domains (Altaf et al., 2007), and methylation of H3K36 that is coupled to transcription elongation by RNA polymerase II (Krebs, 2007). While these marks are generally associated with euchromatin and therefore could facilitate untargeted acetylation, it is somewhat doubtful that they are the principal features of chromatin recognized by untargeted activities. We base this proposition on two observations. The first was reported in studies of cells lacking the Rtf1 subunit of the Paf1 transcriptional elongator complex. Specifically, deletion of *RTF1* blocks overall di- and tri-methylation of H3K4 and di-methylation of H3K79, but does not appear to affect the overall level of acetylation of H3 or H4 measured by immunoblotting of whole cell extracts (Ng et al., 2003). The second, a genome-wide mapping approach, revealed that methylation of H3K36 is restricted to about 25% of genes (Li et al., 2007).

Determining if the type of mechanisms discussed in this section contribute to the interaction of untargeted KAT

complexes with histones during glucose refeed will be the subject of future work (see section 7.7).

7.4 Untargeted KAT activity is controlled by glycolytic metabolism

Our further analysis of glucose regulation of Gcn5 and Esa1 activity revealed a previously unknown physiological mechanism by which histone acetylation is modulated *in vivo*. That is, induction of KAT activity by glucose refeeding in SP depends on increased availability of acetyl-CoA. We base this conclusion on several key observations. The steady state concentration of metabolites required for acetyl-CoA synthesis – acetate and ATP – increases in SP cells within 15 min of glucose feeding. These metabolites are likely used to produce the acetyl-CoA required for histone acetylation, as induction of acetylation depends on enzymes that require acetyl-CoA (the KATs Esa1 and Gcn5) and the enzymes that generate acetyl-CoA (Acs1 or Acs2). Because net production of ATP by glycolysis, and glucose induction of acetate, both require the last step of glycolysis (phosphoenolpyruvate → pyruvate), we propose that glucose-fed SP cells must execute the entire glycolytic program in order to produce acetyl-CoA for use by KATs. This proposal is supported

by the finding that inhibition of Pyk1, which catalyzes the final reaction of glycolysis, strongly dampens glucose induction of H3/H4 acetylation. Cellular energy balance appears to exert rheostat-like control of KAT activity. In the face of glucose depletion, or removal, p^+ cells are able to maintain overall histone acetylation through metabolism of alternative extracellular and intracellular carbon sources. On the other hand, p^0 cells are unable to shift to metabolism of alternate carbon sources and their overall histone acetylation level drops precipitously (Figures 5.7 and 6.1). Further, histone acetylation decreases with increasing amounts of 2-deoxy-D-glucose which depletes ATP, but increases with increasing amounts of glucose (Figure 5.8). Finally, pulse-feeding of glucose causes a transient increase in acetylation, and the duration of peak acetylation depends on the amount of glucose provided to the cells (Figure 3.2 B). The deacetylation of histones at late time points after glucose repletion is likely to be due to a gradual shift in the balance between KAT and HDAC activity in favour of HDACs as the KAT-inducing signal from glucose decays.

While our results reveal that glucose induction of H3/H4 tail acetylation depends on glycolysis and acetyl-CoA synthesis by

the nuclear-localized Acs1 and Acs2 enzymes (Takahashi et al., 2006), they do not rule out indirect contributions of other events in glucose metabolism to induction of H3/H4 acetylation. For example, acetyl-CoA produced by Acs2 in the cytoplasm could be used for fatty acid synthesis which generally improves viability and therefore indirectly supports glucose induction of nuclear KATs (Takahashi et al., 2006). Similarly, metabolic events which depend on mitochondrial acetyl-CoA synthases (Kresnowati et al., 2006) could contribute to steps in physiological reprogramming which are permissive for histone acetylation by nuclear KATs.

Glucose-induction of overall H3/H4 acetylation depends mainly on the untargeted activities of Gcn5 and Esa1 (in SAGA and picNuA4 respectively), and glucose catabolism is required for glucose-induction of overall H3/H4 acetylation; it follows that glucose catabolism controls untargeted KAT activity. This finding has several important implications for our understanding of chromatin regulation during entry into and exit from quiescence. In view of the evidence that acetyl-CoA depletion is an early metabolic event in the transition into SP (Seker et al., 2005), it helps to explain why overall H4 acetylation declines during SP

entry (Ramaswamy et al., 2003; Sandmeier et al., 2002) despite induction of picNuA4 at this time (Boudreault et al., 2003).

Specifically we propose that protein induction of picNuA4 is compatible with overall H4 deacetylation because cells entering SP do not make enough acetyl-CoA to support increased picNuA4 activity. By extension, it is plausible that metabolic regulation of KAT activity is a mechanism for modulating the acetylation state of chromatin even in cells in which nuclear KATs are otherwise fully activated.

Glucose triggers system-wide physiological reprogramming in SP cells: many characteristics of quiescence are lost, hundreds of mRNAs are induced, and events that promote budding are initiated (Granot and Snyder, 1991; Granot and Snyder, 1993; Gray et al., 2004; Liko et al., 2007). Induction of global histone acetylation accompanies these changes (this study), and like them, is a hallmark of re-entry into a proliferative state. This regulation would be beneficial from a fitness perspective in yeast because it intrinsically couples the process of overall chromatin 'activation' to competence for metabolic activity that is associated with a high rate of proliferation. Targeted KATs might also be directly regulated by glycolytic metabolism, although in

SP cells this regulation does not make a substantial contribution to changes in acetylation detected by immunoblotting.

In order to characterize the contribution of untargeted acetylation to glucose regulation of specific chromatin-dependent biochemical activities it would be optimal to obtain mutants in which untargeted activity is compromised. Using such mutants in glucose refeeding experiments, for example, one could determine how the absence of untargeted KAT activity affects the kinetics of gene induction. Although we are attempting to identify untargeted KAT mutants, none has yet been obtained.

In the meantime, we speculate that direct glucose induction of untargeted acetylation in SP cells contributes to transcriptional activation. We favor this possibility based on recent work in yeast. Using an activator bypass approach, Imoberdorf et al. showed that high levels of untargeted H3 acetylation allow robust transcription in the context of weak recruitment of the basal transcription machinery (Imoberdorf et al., 2006). Their work also suggests that the strength of interaction between an activator and its target in the transcriptional machinery might determine the extent to which induction of natural promoters depends on untargeted

acetylation. Therefore, different promoters may display different relative sensitivities to global acetylation due to differences in the strength and number of transcription factors which regulate their transcription. Additionally, transcription of genes with promoters that are constitutively bound by transcription factors are more likely to be strongly affected by global histone acetylation level than are genes regulated by transcription factors that only associate in response to certain stimuli. We predict that glycolytic induction of untargeted acetylation in SP cells helps to overcome the gene induction barrier presented by low global acetylation in G0 (Ramaswamy et al., 2003; Sandmeier et al., 2002). When SP cells are refed all the nutrients required for proliferation (glucose, amino acids, vitamins, etc.), the full spectrum of genes that must be induced for a balanced physiological response to the growth stimulus will benefit from chromatin opening triggered by glucose.

7.5 Acetylation of nonhistone proteins

Notably, histones are not the only proteins which a subject to acetylation. A recent study in yeasts that used protein microarrays to identify nonhistone substrates of the NuA4 complex identified 13 proteins which appear to be in vivo

substrates(Lin et al., 2009). Additionally many mammalian nonhistone proteins are known to be regulated by acetylation including proteins involved in nutrient metabolism such as AceCS1 and AceCS2, the homologues of the yeast acetyl-CoA synthases (Hallows et al., 2006; Schwer et al., 2006) and numerous proteins which are involved in transcriptional processes (Sterner and Berger, 2000) . How the interaction between these acetylated proteins and the factors which acetylate or deacetylate them will dictate what effect fluctuation in cellular acetyl-CoA levels have upon their acetylation state. Proteins which continually interact with acetylases and deacetylases may show regulation of acetylation similar to that seen for histones. On the other hand proteins which interact with (de)acetylases only in response to particular stimuli may react quite differently. Tubulin, for example, is highly acetylated in glucose starved cells and its level of acetylation actually drops after glucose refeeding(Campetelli et al., 2005).

7.6 Beyond yeast

Untargeted KAT activity almost certainly contributes to chromatin acetylation in higher organisms (Nagy and Tora, 2007). The HBO1 and PCAF/STAGA/TFTC complexes of human

are respectively equivalent to picNuA4 and SAGA of yeast (Lee and Workman, 2007). HBO1, like picNuA4, is specialized for untargeted acetylation of nucleosomal H4 (Doyon et al., 2006), and the human SAGA-related enzymes are capable of untargeted acetylation of nucleosomal H3 in vitro (Brand et al., 1999; Martinez et al., 2001; Ogryzko et al., 1998)

What we have learned in yeast suggests that direct metabolic regulation of untargeted KATs probably manifests itself in cells which depend heavily on glycolysis for their metabolic needs. Such cells include T cells and cancer cells. T cells, like yeast, can be triggered to re-enter proliferation from a non-dividing state in which metabolism is relatively low. Intriguingly, this T cell 'activation' requires induction of glycolytic metabolism and (at least in vitro) is associated with replication-independent induction of overall H4 tail acetylation (Jacobs et al., 2008; Taplick et al., 1998). We speculate induction of histone acetylation in T cells partly involves glycolytic upregulation of untargeted KAT activity.

Many cancer cell types are specialized for energy generation by glycolysis (Deberardinis et al., 2008). Cancer cells likely experience substantial fluctuations in glucose availability during

vascular remodelling within solid tumors and during egress from poorly vascularised tumors (Gillies et al., 2008). Perhaps fluctuations in glycolysis accompany such changes in access to glucose and directly modulate untargeted KAT activity, thereby affecting transcriptional reprogramming that promotes cancer cell survival. What makes this scenario attractive is the evidence that the glycolytic phenotype of cancer cells, and tumor cell growth in vivo, depend on expression of pyruvate kinase isoform PKM2 (Christofk et al., 2008). PKM2 is the human equivalent of yeast Pyk1, the glycolytic enzyme required for glucose induction of global acetylation in G0.

7.7 Signaling defects displayed by cells lacking mtDNA

Our transcriptional profiling of p^+ and p^0 cells before and after removal of glucose from their media revealed that p^0 cells display defects in establishing the normal transcriptional response to glucose removal. Specifically, in response to glucose deprivation, p^0 cells fail to upregulate expression of genes that are induced by Snf1 activity and genes which are repressed by PKA activity. As noted earlier, p^0 cells rapidly lose overall histone acetylation when glucose is removed from their media.

While the diminished level of histone acetylation in p^0 cells may play some role in their transcriptional defects, our analyses have revealed clear aberrations in components of the signaling cascades which normally mediate transcriptional responses to glucose deprivation. Currently, we suspect that the transcriptional defects displayed by p^0 cells are primarily the result of an inability to fully inactivate PKA in response to glucose removal. Activation of Snf1 via phosphorylation of Thr210 in its activation loop appears to occur normally in p^0 cells (Figure 6.2A). In p^+ cells, both Msn2 and Ume6 are hyperphosphorylated in response to removal of glucose (Garreau et al., 2000; Xiao and Mitchell, 2000) (Figure 6.3 A and B). Downregulation of PKA activity is required for this to occur (Garreau et al., 2000; Rubin-Bejerano et al., 1996). In p^0 cells, neither Msn2 nor Ume6 undergo hyperphosphorylation in response to glucose removal (Figure 6.3 A and B). This suggests that PKA activity may be misregulated in p^0 cells.

Regulatory defects in control of the yeast Ras proteins, which activate PKA through their control of adenylate cyclase activity, may account for this. (Wang and Deschenes, 2006) noted that Ras2 relocalizes to the mitochondrial membrane when

cells are treated with sodium azide. Our results suggest that p^0 cells may display abnormal regulation of the Ras proteins.

Decreasing Ras activity by deleting *RAS2* seems to allow some phosphorylation of Ume6 (Figure 6.6 A) and Ras2 appears to be modified, potentially by phosphorylation, in response to glucose removal in p^+ but not p^0 cells. Together these results support the hypothesis that misregulation of PKA activity in p^0 cells accounts for a large portion of the defects detected by our transcriptional profiling experiments.

Our results suggest a potential mechanism behind the known requirement for respiration during meiosis/sporulation. Diploid yeast undergo meiosis to form spores which can survive extended periods of starvation. The transcriptional activator Ime1 serves as the master regulator of meiosis. Respiration is required for expression of Ime1 (Treinin and Simchen, 1993). The expression of Ime1 is blocked in p^0 cells, nuclear petite mutants and cells treated with the cytochrome C oxidase inhibitor sodium azide (Jambhekar and Amon, 2008; Treinin and Simchen, 1993). Ime1 expression is repressed during growth on glucose by Sok2. This repression depends upon PKA phosphorylation of Sok2 at Thr598 (Shenhar and Kassir, 2001).

Activation of Ime1 expression is mediated by the transcription factors Msn2 and Msn4 which are repressed by PKA activity (Shenhar and Kassir, 2001). Obviously an inability to inactivate PKA after removal of glucose would account for the lack of Ime1 expression in p^0 cells.

The defects in phosphorylation of Ume6 which we see in p^0 cells could also contribute to defects in meiotic gene expression downstream of Ime1 expression. During vegetative growth Ume6 acts as a repressor at the promoters of early meiotic genes (Rubin-Bejerano et al., 1996; Williams et al., 2002). During meiosis Ume6 is phosphorylated by the GSK β homologue Rim11 (Malathi et al., 1997; Rubin-Bejerano et al., 1996; Xiao and Mitchell, 2000). This switches the activity of Ume6 from repressor to activator. Phosphorylated Ume6 interacts with Ime1 to drive expression of the early meiotic genes (Xiao and Mitchell, 2000). Therefore if signaling defects similar to those seen in haploid p^0 cells occur in diploid p^0 cells, it could account for some of the meiotic defects that result from inhibition of respiration.

7.8 Future directions

The relative contributions of untargeted and targeted histone acetylation to initiation of a new transcriptional program are still unclear. Furthermore the nature of the interactions between untargeted KAT complexes and chromatin are largely uncharacterized. Additionally, we have not analyzed all sites of acetylation on histones, or tested if modifications at particular sites are regulated by different signaling pathways. Therefore, future investigations of control of histone acetylation will 1) explore the role of components of KAT complexes in regulation of global acetylation, 2) examine the transcriptional effects of increasing global acetylation in the absence of targeted KAT activity, 3) test if certain site specific acetylation events that occur after glucose refeed do depend upon classical signalling pathways. These investigations can be pursued as follows:

1) Investigate the role of interactions between components of KAT complexes and modified histones (discussed in section 7.3) on the control of untargeted acetylation during glucose refeed. The role of individual KAT/HDAC complex components can be evaluated in deletion mutants. In strains in which protein components of these complexes are essential for normal

viability, the protein of interest could be fused to a heat inducible degron allowing for their rapid depletion (Kanemaki et al., 2003; Sanchez-Diaz et al., 2004). Analysis of the domains within these proteins, which are required for glucose refeed, along with analysis of the molecules with which they interact, will markedly improve our understanding of untargeted acetylation.

2) Investigate the transcriptional response to increasing global acetylation when targeting of SAGA/SLIK and NuA4 is disrupted. Tra1 is a component of SAGA/SLIK and NuA4. Inactivation of Tra1 by heat shock in the *tra1-2* mutant allows increases in global acetylation without targeting of these complexes to transcriptional activators. Our *tra1-2/chd1Δ* mutants will also be employed in this analysis, to eliminate targeting of the SAGA/SLIK complex to H3 tri-me K4 at promoter regions.

3) Approximately 90% of the transcriptional response to glucose can be recapitulated by activation of PKA or overexpression of Sch9 (Wang et al., 2004; Zaman et al., 2009). We will therefore evaluate whether PKA or Sch9 activity is required for particular site specific acetylations on H3 and H4. For this purpose, we will use strains harbouring analogue sensitive forms of PKA or Sch9, or both PKA and Sch9, in experiments conducted as described

for Figure 4.3 B. Western analysis will employ anti-bodies specific for individual sites of acetylation on H3 and H4.

The mechanisms interfering with the transcriptional response to glucose removal in p^0 cells will be investigated as follows:

1) Subcellular location of Ras2 and its GTP loading state in p^0 and p^+ cells during log growth and after depletion or removal of glucose will be evaluated. Immunofluorescence microscopy can be used to follow localization of GFP tagged versions of Ras2. In their GTP bound state, the mammalian Ras proteins interact with Raf1 through its Ras binding domain (RBD). Immobilized versions of the Raf1 RBD have been used successfully to analyze cellular Ras GTP levels (Colombo et al., 2004; Rudoni et al., 2001; Taylor and Shalloway, 1996).

2) Evaluate phosphorylation of Msn2, Ume6 and Ras2 activation state in diploid p^0 and p^+ cells under conditions that induce sporulation.

7.9 References

- Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L., Workman, J.L. and Cote, J. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *Embo J*, **18**, 5108-5119.
- Altaf, M., Utley, R.T., Lacoste, N., Tan, S., Briggs, S.D. and Cote, J. (2007) Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. *Mol Cell*, **28**, 1002-1014.
- Berndsen, C.E., Tsubota, T., Lindner, S.E., Lee, S., Holton, J.M., Kaufman, P.D., Keck, J.L. and Denu, J.M. (2008) Molecular functions of the histone acetyltransferase chaperone complex Rtt109-Vps75. *Nat Struct Mol Biol*.
- Boudreault, A.A., Cronier, D., Selleck, W., Lacoste, N., Utley, R.T., Allard, S., Savard, J., Lane, W.S., Tan, S. and Cote, J. (2003) Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev*, **17**, 1415-1428.
- Brand, M., Yamamoto, K., Staub, A. and Tora, L. (1999) Identification of TATA-binding protein-free TAFII-containing complex subunits suggests a role in nucleosome acetylation and signal transduction. *J Biol Chem*, **274**, 18285-18289.
- Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S. and Workman, J.L. (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science*, **292**, 2333-2337.
- Campetelli, A.N., Previtali, G., Arce, C.A., Barra, H.S. and Casale, C.H. (2005) Activation of the plasma membrane H-ATPase of *Saccharomyces cerevisiae* by glucose is mediated by dissociation of the H(+)-ATPase-acetylated tubulin complex. *Febs J*, **272**, 5742-5752.
- Carrozza, M.J., Florens, L., Swanson, S.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005) Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. *Biochim Biophys Acta*, **1731**, 77-87; discussion 75-76.
- Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L. and Cantley, L.C. (2008) The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*, **452**, 230-233.
- Colombo, S., Ronchetti, D., Thevelein, J.M., Winderickx, J. and Martegani, E. (2004) Activation state of the Ras2 protein and glucose-induced signaling in *Saccharomyces cerevisiae*. *J Biol Chem*, **279**, 46715-46722.
- Deberardinis, R.J., Sayed, N., Ditsworth, D. and Thompson, C.B. (2008) Brick by brick: metabolism and tumor cell growth. *Curr Opin Genet Dev*, **18**, 54-61.
- Doyon, Y., Cayrou, C., Ullah, M., Landry, A.J., Cote, V., Selleck, W., Lane, W.S., Tan, S., Yang, X.J. and Cote, J. (2006) ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Mol Cell*, **21**, 51-64.
- Garreau, H., Hasan, R.N., Renault, G., Estruch, F., Boy-Marcotte, E. and Jacquet, M. (2000) Hyperphosphorylation of Msn2p and Msn4p in

- response to heat shock and the diauxic shift is inhibited by cAMP in *Saccharomyces cerevisiae*. *Microbiology*, **146** (Pt 9), 2113-2120.
- Gillies, R.J., Robey, I. and Gatenby, R.A. (2008) Causes and consequences of increased glucose metabolism of cancers. *J Nucl Med*, **49 Suppl 2**, 24S-42S.
- Granot, D. and Snyder, M. (1991) Glucose induces cAMP-independent growth-related changes in stationary-phase cells of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*, **88**, 5724-5728.
- Granot, D. and Snyder, M. (1993) Carbon source induces growth of stationary phase yeast cells, independent of carbon source metabolism. *Yeast*, **9**, 465-479.
- Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev*, **11**, 1640-1650.
- Gray, J.V., Petsko, G.A., Johnston, G.C., Ringe, D., Singer, R.A. and Werner-Washburne, M. (2004) "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev*, **68**, 187-206.
- Grubisha, O., Smith, B.C. and Denu, J.M. (2005) Small molecule regulation of Sir2 protein deacetylases. *Febs J*, **272**, 4607-4616.
- Hallows, W.C., Lee, S. and Denu, J.M. (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc Natl Acad Sci U S A*, **103**, 10230-10235.
- Imoberdorf, R.M., Topalidou, I. and Strubin, M. (2006) A role for gcn5-mediated global histone acetylation in transcriptional regulation. *Mol Cell Biol*, **26**, 1610-1616.
- Jacobs, S.R., Herman, C.E., Maciver, N.J., Wofford, J.A., Wieman, H.L., Hammen, J.J. and Rathmell, J.C. (2008) Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol*, **180**, 4476-4486.
- Jambhekar, A. and Amon, A. (2008) Control of meiosis by respiration. *Curr Biol*, **18**, 969-975.
- Kanemaki, M., Sanchez-Diaz, A., Gambus, A. and Labib, K. (2003) Functional proteomic identification of DNA replication proteins by induced proteolysis in vivo. *Nature*, **423**, 720-724.
- Katan-Khaykovich, Y. and Struhl, K. (2002) Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes Dev*, **16**, 743-752.
- Krebs, J.E. (2007) Moving marks: dynamic histone modifications in yeast. *Mol Biosyst*, **3**, 590-597.
- Kresnowati, M.T., van Winden, W.A., Almering, M.J., ten Pierick, A., Ras, C., Knijnenburg, T.A., Daran-Lapujade, P., Pronk, J.T., Heijnen, J.J. and Daran, J.M. (2006) When transcriptome meets metabolome: fast cellular responses of yeast to sudden relief of glucose limitation. *Mol Syst Biol*, **2**, 49.
- Kuo, M.H., vom Baur, E., Struhl, K. and Allis, C.D. (2000) Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. *Mol Cell*, **6**, 1309-1320.

- Ladurner, A.G. (2006) Rheostat control of gene expression by metabolites. *Mol Cell*, **24**, 1-11.
- Lee, K.K. and Workman, J.L. (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol*, **8**, 284-295.
- Li, B., Gogol, M., Carey, M., Lee, D., Seidel, C. and Workman, J.L. (2007) Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. *Science*, **316**, 1050-1054.
- Liko, D., Slattery, M.G. and Heideman, W. (2007) Stb3 binds to ribosomal RNA processing element motifs that control transcriptional responses to growth in *Saccharomyces cerevisiae*. *J Biol Chem*, **282**, 26623-26628.
- Lin, Y.Y., Lu, J.Y., Zhang, J., Walter, W., Dang, W., Wan, J., Tao, S.C., Qian, J., Zhao, Y., Boeke, J.D., Berger, S.L. and Zhu, H. (2009) Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. *Cell*, **136**, 1073-1084.
- Malathi, K., Xiao, Y. and Mitchell, A.P. (1997) Interaction of yeast repressor-activator protein Ume6p with glycogen synthase kinase 3 homolog Rim11p. *Mol Cell Biol*, **17**, 7230-7236.
- Martinez, E., Palhan, V.B., Tjernberg, A., Lyman, E.S., Gamper, A.M., Kundu, T.K., Chait, B.T. and Roeder, R.G. (2001) Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. *Mol Cell Biol*, **21**, 6782-6795.
- Nagy, Z. and Tora, L. (2007) Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene*, **26**, 5341-5357.
- Ng, H.H., Dole, S. and Struhl, K. (2003) The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J Biol Chem*, **278**, 33625-33628.
- Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.J., Howard, B.H., Qin, J. and Nakatani, Y. (1998) Histone-like TAFs within the PCAF histone acetylase complex. *Cell*, **94**, 35-44.
- Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd and Grant, P.A. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature*, **433**, 434-438.
- Ramaswamy, V., Williams, J.S., Robinson, K.M., Sopko, R.L. and Schultz, M.C. (2003) Global control of histone modification by the anaphase-promoting complex. *Mol Cell Biol*, **23**, 9136-9149.
- Reid, J.L., Iyer, V.R., Brown, P.O. and Struhl, K. (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol Cell*, **6**, 1297-1307.
- Rubin-Bejerano, I., Mandel, S., Robzyk, K. and Kassir, Y. (1996) Induction of meiosis in *Saccharomyces cerevisiae* depends on conversion of the transcriptional repressor Ume6 to a positive regulator by its regulated association with the transcriptional activator Ime1. *Mol Cell Biol*, **16**, 2518-2526.
- Rudoni, S., Colombo, S., Coccetti, P. and Martegani, E. (2001) Role of guanine nucleotides in the regulation of the Ras/cAMP pathway in *Saccharomyces cerevisiae*. *Biochim Biophys Acta*, **1538**, 181-189.
- Sanchez del Pino, M.M., Lopez-Rodas, G., Sendra, R. and Tordera, V. (1994) Properties of the yeast nuclear histone deacetylase. *Biochem J*, **303** (Pt 3), 723-729.

- Sanchez-Diaz, A., Kanemaki, M., Marchesi, V. and Labib, K. (2004) Rapid depletion of budding yeast proteins by fusion to a heat-inducible degron. *Sci STKE*, **2004**, PL8.
- Sandmeier, J.J., French, S., Osheim, Y., Cheung, W.L., Gallo, C.M., Beyer, A.L. and Smith, J.S. (2002) RPD3 is required for the inactivation of yeast ribosomal DNA genes in stationary phase. *Embo J*, **21**, 4959-4968.
- Schwer, B., Bunkenborg, J., Verdin, R.O., Andersen, J.S. and Verdin, E. (2006) Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc Natl Acad Sci U S A*, **103**, 10224-10229.
- Seker, T., Moller, K. and Nielsen, J. (2005) Analysis of acyl CoA ester intermediates of the mevalonate pathway in *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol*, **67**, 119-124.
- Shahbazian, M.D. and Grunstein, M. (2007) Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem*, **76**, 75-100.
- Shenhar, G. and Kassir, Y. (2001) A positive regulator of mitosis, Sok2, functions as a negative regulator of meiosis in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **21**, 1603-1612.
- Sklenar, A.R. and Parthun, M.R. (2004) Characterization of yeast histone H3-specific type B histone acetyltransferases identifies an ADA2-independent Gcn5p activity. *BMC Biochem*, **5**, 11.
- Sterner, D.E. and Berger, S.L. (2000) Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev*, **64**, 435-459.
- Takahashi, H., McCaffery, J.M., Irizarry, R.A. and Boeke, J.D. (2006) Nucleocytosolic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription. *Mol Cell*, **23**, 207-217.
- Taplick, J., Kurtev, V., Lager, G. and Seiser, C. (1998) Histone H4 acetylation during interleukin-2 stimulation of mouse T cells. *FEBS Lett*, **436**, 349-352.
- Taylor, S.J. and Shalloway, D. (1996) Cell cycle-dependent activation of Ras. *Curr Biol*, **6**, 1621-1627.
- Tong, J.K., Hassig, C.A., Schnitzler, G.R., Kingston, R.E. and Schreiber, S.L. (1998) Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. *Nature*, **395**, 917-921.
- Treinin, M. and Simchen, G. (1993) Mitochondrial activity is required for the expression of IME1, a regulator of meiosis in yeast. *Curr Genet*, **23**, 223-227.
- Venkatasubrahmanyam, S., Hwang, W.W., Meneghini, M.D., Tong, A.H. and Madhani, H.D. (2007) Genome-wide, as opposed to local, antisilencing is mediated redundantly by the euchromatic factors Set1 and H2A.Z. *Proc Natl Acad Sci U S A*, **104**, 16609-16614.
- Vogelauer, M., Wu, J., Suka, N. and Grunstein, M. (2000) Global histone acetylation and deacetylation in yeast. *Nature*, **408**, 495-498.
- Wang, G. and Deschenes, R.J. (2006) Plasma membrane localization of Ras requires class C Vps proteins and functional mitochondria in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **26**, 3243-3255.
- Wang, Y., Pierce, M., Schnepfer, L., Guldal, C.G., Zhang, X., Tavazoie, S. and Broach, J.R. (2004) Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. *PLoS Biol*, **2**, E128.
- Williams, R.M., Primig, M., Washburn, B.K., Winzeler, E.A., Bellis, M., Sarrauste de Menthieri, C., Davis, R.W. and Esposito, R.E. (2002) The

C12332